Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Chemical composition and evaluation of antibacterial activity of fennel (*Foeniculum vulgare* Mill) seed essential oil against some pathogenic bacterial strains

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ABSTRACT

The aim of this work is to assess the *in vitro* antibacterial activity of the extracted essential oil (EO) obtained from dry seeds of fennel, *Foeniculum vulgare* Mill, collected from Meknes, (Morocco), against seven pathogenic bacteria: *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus epidermidis* and *Staphylococcus aureus*. The extraction of EO from fennel was performed by hydro-distillation in the Clevenger-type device. The yield was close to 2.82%. The identification of the chemical composition of fennel EO by gas chromatography coupled with mass spectrometry (GC/MS), has given 25 constituents. They represent 97.525% of all constituents existing in the essential oil. The major compound was the trans-anethole (with 44.376%). The result of this study showed that the fennel EO has a remarkable inhibitory activity against the majority of the examined microorganisms, especially against *A. baumannii*, with the exception of *P. aeruginosa* and *E. coli*, as compared to three standard antibiotics. It also exhibited a strong antimicrobial activity against *A. baumannii* (with a growth inhibition zone of 26 mm) compared to the standard antibiotics examined, with minimum inhibitory concentration (MIC) of 1/2000 (v/v) and *S. aureus* (growth inhibition zone of 20 mm) with MIC of 1/10000 (v/v). These results indicate that the fennel OE examined represents a potential source of natural antibacterial substances which can be used against pathogenic strains.

Keywords: *Foeniculum vulgare* Mill, Essential oil, Chemical composition, Inhibitory activity, MIC.

INTRODUCTION

The essential oils are known by their antimicrobial activity and some are classified as substances that are safe and could, therefore, be used to prevent the growth of pathogenic microorganisms and contaminants (Gachkar *et al*. 2007; Dehghan *et al*. 2020; Fancello *et al*. 2020; Hasheminya *et al*. 2020; Khezri *et al*. 2020; Ruiz-Rico *et al*. 2020). Hence, the last decade witnessed an increase in the number of investigations on plants as a source of human disease management (Aiyelagabe 2001, Woldemichael *et al*. 2003). In Morocco, infectious diseases

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research

constitute a public health problem because of their frequency and their severity (Bourgeois 1999). Due to the lack of health care, the high price of drugs and people's socio-cultural habits, many developing countries cannot buy modern drugs, so they are obligated to search for other alternative products that are proved to be safe, economical and culturally acceptable (Adefa *et al*. 2011). For that reason, over 70% of the world's population use herbal remedies for their primary health care system (Ekor 2014). Plants have formed and still form the basis of many therapies in several countries, and many active ingredients have been found in medicinal plants. In addition, many publications are also interested in the research and development of alternatives to multiple resistance to antibiotics. However, these alternatives are not all of equivalent quality and do not necessarily have a similar activity to that of antibiotics. Despite the proven efficiency of these antibiotics in the prevention and outbreak control of infectious diseases, their repeated applications have resulted in the ineffective and acquisition of microbial resistance to the applied antibiotics and their unpleasant side effects on human health. (Bialonska *et al*. 2010).

Fennel, *Foeniculum vulgare* Mill is one of the most popular medicinal plants in Morocco. It belongs to the Apiaceae family and generally grows in many parts of the world (Raal *et al*. 2011). The different parts of this plant meet several uses. Fruits and essential oils of ripe fennel are used in cosmetics and pharmaceutical products. They are also used as flavoring agents in food products such as liqueurs, bread, pickles, pastries and cheese (Telci *et al*. 2009, Zoubiri *et al*. 2014). In addition, leaves and seeds are used in many culinary traditions (Ehsanipour *et al*. 2012). Furthermore, the fennel is also used in the treatment of renal calculi, menopausal problems, nausea, obesity, and also it stimulates the appetite and facilitates digestion (Zahid *et al*. 2009).

The objective of this work is to identify the chemical composition and to highlight the antibacterial activity of the fennel essential oil against six pathogenic bacterial strains and to determine the values of their MIC and MBC.

MATERIALS AND METHODS

Plant material

The samples of the *Foeniculum vulgare* Mill seeds have been harvested in June 2014 during flowering stage in the region of Meknes, located in the north of Morocco in altitude of 552 meters above mean sea level (MAMSL). The samples have been previously dried in the shade during the fifteen days to get ready for storage.

Extraction of the essential oil

The hydro-distillation of fennel seeds was accomplished using a Clevenger-type apparatus (Clevenger 1928). Triple repeated weighing of 300 g of the dried fennel seeds were added to 2-L distilled water into a 5-L flask. The whole was heated until boiling for 5 h. The essential oil, after their extraction, was collected and dried with anhydrous sodium sulphate, then recovered and stored in a small bottle opaque at 4 °C in the dark, (Na_2SO_4), until being analysed.

Calculating the essential oil yield

The essential oil yield is the ratio between the weight of the oil extracted and the weight of the plant to treat (Carré 1953). The yield expressed in percentage is calculated by the following formula (AFNOR 1986):

$$Y = W_X / W_Y \, 100$$

- Y : Yield of the oil in percentage
- Wx : Net weight of the oil in grams
- Wy : Total weight of the plant in grams

Gas chromatography-mass spectrometry (GC/MS) analysis

The chromatographic analysis of the essential oil was carried out using gas chromatography type Perkin Elmer Clarus® 580 coupled to a mass spectrometer type Perkin Elmer Clarus® SQ 8 S. The fragmentation was performed using electronic impact under the ionization energy of 70 eV, with a column Rxi®-5ms (phase of low polarity, Crossbond®-bond 5% diphenyl/ 95% dimethyl polysiloxane) of 30 m in length, internal diameter equal

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research

to 0.25 mm and the thickness of the film was 0.25 µm. The programming of the temperature was 50 °C for 2 min followed by a rise of 8 °C/min for 18 min to reach 290 °C, the gas vector was helium with a flow of 1 mL/min. Injection mode was the mode split. The volume of the sample injected was 1 µL of the essential oil diluted in hexane. The identification of the different constituents was carried out through the comparison of their mass spectra with those of the reference products contained in the Computerized libraries available: NIST/EPA/NIH Mass Spectral Library Search (version 2.0 g) 2011, Wiley Registry of mass spectral data as well as those of the basis of spectral data Adams (Adams 2001).

Microbial material

The microbial strains were isolated from the patients in the Medical Analysis Laboratory, El Idrissi Hospital, Kenitra, Morocco. In this study, the seven microorganisms used were *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus epidermidis* and *Staphylococcus aureus*, obtained from several infections (urinary, intestinal, respiratory, etc.) (Table 1). They were maintained by transplanting on nutrient agar favourable for their growth for 24 h, in the dark at 37 °C, to obtain isolated young colonies used to prepare the inoculums.

Table 1. Pathogenic bacteria used in the test (UTI: Urinary Tract Infection; SI: Skin Infection)

Microbial group	Tested strains	Origin of strain
Gram-negative	*E. coli*	UTI
Enterobacteriaceae	*K. pneumoniae*	UTI
	E. cloacae	UTI
Gram-negative not	*P. aeruginosa*	SI
Enterobacteriaceae	*A. baumannii*	UTI
Gram-positive bacteria	*S. aureus*	UTI
	S. epidermidis	UTI

Antibacterial activity

The antimicrobial activity assay was performed by the agar diffusion test (NCCLS 1997). Mueller–Hinton Agar (MHA) was used as the culture medium for the bacteria in this assay. (Leyral & Joffin 2001). Briefly, the sterile dishes were inoculated using 1000 µL of a suspension containing 10^8 CFU/mL of bacteria (Bekhechi *et al*. 2007). The sterile Whatman paper discs of 6 mm in diameter were soaked with 5-µL pure oil, then deposited on the MHA surfaces, which were inoculated by the strains examined in advance. After remaining at 4 °C for 2 h, these Petri dishes were incubated at 37 °C for 24 h (Gachkar *et al*. 2007). The effect of the antibacterial activity on the germs is evidenced by the appearance of the inhibition zones. The zone of inhibition is considered to be the clear halo around discs where there is a complete absence of growth. Thus, the sensitivity of bacteria to essential oil is appreciated by measuring the inhibition zone diameter in millimetres. Rifampicin (RA_{30}), Netilmicin (NET_{30}) and Levofloxacin (LVX_5) were used as positive controls. These choices were due to the sensitivity of the strains chosen for these antibiotics.

Determination of the minimum inhibitory concentration (MIC)

The MIC of the essential oil was applied according to the technique described by Remmal and Satrani (Remmal *et al*. 1993; Satrani *et al*. 2001). Due to the immiscibility of the essential oils in water and thus, in the culture medium, emulsification was achieved in a 0.2% agar solution. Dilutions of agar solution were prepared at 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500 (v/v). In test tubes, each having 13.5 mL of solid medium MHA (Muller Hinton Agar), sterilized by autoclaving at 121°C for 20 min and cooled at 45°C. 1.5 mL of each of the dilutions of agar were added aseptically in order to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000 and 1/5000 (v/v). The tubes were then vortexed before being poured into sterile Petri dishes. The seeding was performed by streaking with platinum loop calibrated to take the same volume of inoculum. The latter is taken from a young culture of 18 h at 37 °C in broth. The solid medium MHA

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
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supplemented with the 0.2 % agar solution alone was also prepared and used as a negative control. Each assay was repeated three times.

Determination of the minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is the lowest concentration in which the antimicrobial samples (e.g. essential oil) will kill 99.9% of the bacterial strains examined. It was determined by the transplantation, presumed negative cultures in the MIC test, with the help of a cove in the middle of Muller Hinton agar. After incubation, where there was no growth of microbes corresponded to destructive concentrations of the bacterial cell (Esmail *et al*. 2015).

RESULTS

Yield of essential oil (EO)

The essential oil (EO) yield of the examined fennel, *Foeniculum vulgare* Mill was 2.82 %. We obtained higher yield compared to that reported by Mata *et al*. (0.1 %) (Mata *et al*. 2007).

Chemical composition of the *F. vulgare* essential oil

The CG/MS analysis of the fennel seed EO has identified the presence of 25 compounds, corresponding to a total of approximately 97.525% of identified compounds presented in Table 3. According to the results obtained from Table 3 and Fig. 1, the fennel seeds essential oil was composed mainly of Trans-anethole (44.376%), 1-Butanone, 2-chloro-3-methyl-1-[4-(1-methylethyl) phenyl]- (19.411%), L-Fenchone (11.728%), D- limonene (8.287%) and the Estragole (5.092%). Other compounds were also present but at low levels: p-anisaldehyde (2.226%), α- pinene (1.827 %), and β-myrcene (1.135%).

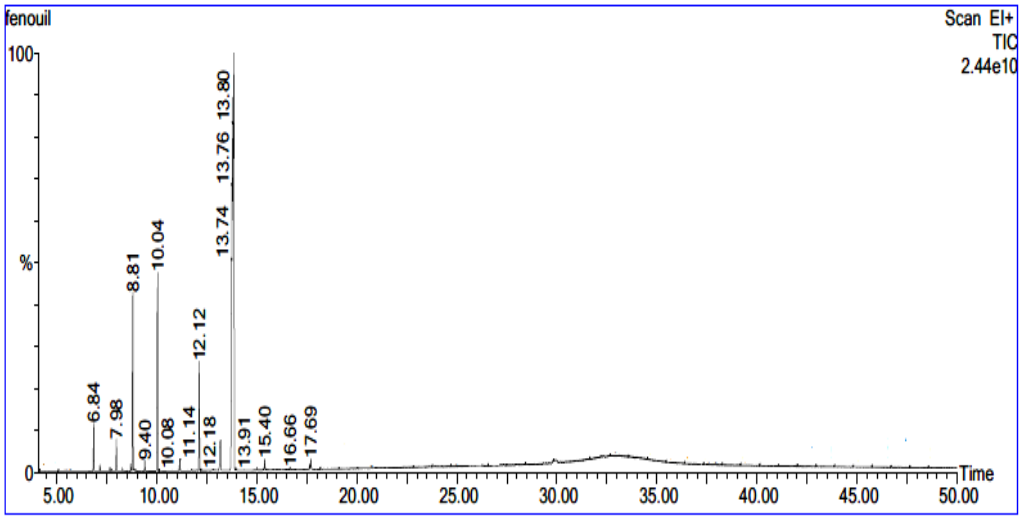

Fig. 1. Chromatogram of *F. vulgare* seed volatile EO obtained by GC/MS. The horizontal axis: Retention time (RT), the vertical axis: composition rate (%).

Antibacterial activity

The results of the antibacterial activity are indicated in Table 4, exhibiting that the fennel EO exercises a very pronounced power of antibacterial on the strains of *A. baumannii*, *S. aureus* and *S. epidermidis*. The inhibition diameters were in the descending order of 26 mm, 20 mm and 16 mm respectively. The three other strains *K. pneumoniae*, *E. cloacae*, *E. coli*, proved to be sensitive, but with a lesser degree, compared to the two aforementioned bacteria. The obtained inhibition diameters of the second group were 14 mm, 10 mm, 8 mm respectively. The *P. aeruginosa* displayed a resistance to the fennel EO (0 mm). Furthermore, the results obtained by the growth inhibition zone test (Table 4) showed that standard antibiotics exhibit variable responses to the different strains assayed. The fennel EO was the most active to inhibit the *A. baumannii* in comparison

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DOI: 10.22124/cjes.2020.4276

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with the three standard antibiotics examined. However, the Rifampicin showed a comparable activity such as the fennel EO against *E. cloacae*, *K. pneumoniae* and *S. epidermidis* with inhibition zone diameters in the range of 10 to 16 mm. On the other hand, Levofloxacin revealed a higher sensitivity against *P. aeruginosa* and *E. coli*, while the fennel EO was ineffective. However, in the case of *S. aureus*, both the fennel EO and the three standard antibiotics were completely effective.

Table 3. Chemical constituents of the *F. vulgare* essential oil analysed by GC/MS.

Peak	Compounds	Formula	RT	%
1	α-pinene	$C_{10}H_{16}$	6.838	1.827
2	Camphene	$C_{10}H_{16}$	7.151	0.272
3	β-Phellandrene	$C_{10}H_{16}$	7.655	0.137
4	β-pinene	$C_{10}H_{16}$	7.734	0.084
5	β-myrcene	$C_{10}H_{16}$	7.98	1.135
6	α-Phellandrene	$C_{10}H_{16}$	8.285	0.128
7	p-Cymene	$C_{10}H_{14}$	8.698	0.269
8	D-limonene	$C_{10}H_{16}$	8.806	8.287
9	Eucalyptol	$C_{10}H_{18}O$	8.856	0.067
10	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	$C_{10}H_{16}$	8.931	0.092
11	γ-Terpinen	$C_{10}H_{16}$	9.398	0.389
12	L-Fenchone	$C_{10}H_{16}O$	10.044	11.728
13	Camphor	$C_{10}H_{18}O$	11.141	0.401
14	Terpinene-4-ol	$C_{10}H_{18}O$	11.749	0.094
15	Estragole	$C_{10}H_{12}O$	12.124	5.092
16	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1S-exo)-	$C_{12}H_{20}O_2$	12.804	0.072
17	p-Anisaldehyde	$C_8H_8O_2$	13.175	2.226
18	Trans –anethole	$C_{10}H_{12}O$	13.805	44.376
19	1-Butanone, 2-chloro-3-methyl-1-[4-(1-methylethyl)phenyl]-	$C_{14}H_{19}ClO$	13.846	19.411
20	4-[(S)-sec-Butyl]anisole	$C_{11}H_{16}O$	14.997	0.178
21	4-Methoxyphenylacetone	$C_{10}H_{12}O_2$	15.326	0.070
22	Formic acid, 2-isopropylphenyl ester	$C_{10}H_{12}O_2$	15.397	0.448
23	m-Anisic acid, 4-chlorophenyl ester	$C_{14}H_{11}ClO_3$	16.656	0.118
24	Myristicin	$C_{11}H_{12}O_3$	17.632	0.148
25	1,3-Benzenediamine, N,N,N',N'-tetramethyl	$C_{10}H_{16}N_2$	17.69	0.476
Total				97.525%

Determination of minimum inhibitory concentration (MIC)

The antimicrobial activity was assessed by observing the inhibition power of EO extracted from *F. vulgare* at different concentrations on the bacteria examined. The minimum inhibitory concentration (MIC) is the lowest concentration of the essential oil that inhibits any visible culture of a bacterial strain after 18 h of incubation at 37 °C.

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
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According to the results obtained, the fennel seed EO exerted a strong antibacterial activity against *A. baumannii* with a MIC 1/2000 v/v, followed by *S. aureus* and *S. epidermidis*, both shared the same MIC of 1/1000 v/v, and *K. pneumonia* with a MIC of 1/250 v/v , while the lowest activity was against *E. cloacae* with a MIC of 1/100 v/v. However, *E. coli* and *P. aeruginosa* resisted all the concentrations of our fennel seed EO (Table 5, Fig. 2).

Table 4. Antibacterial effects of fennel seed oil against the pathogenic bacteria monitored by the disk diffusion assay (-: No zone of inhibition; NT: Not Tested; EOFv: Fennel EO; RA_{30}: Rifampicin (30 µg); NET_{30}: Netilmicin (30 µg); LVX_5: Levofloxacin (5 µg)).

	The inhibition zone diameters in mm of the strains examined						
	Gram-negative bacteria					**Gram-positive bacteria**	
	E. coli	***E. cloacae***	***K. pneumoniae***	***P. aeruginosa***	***A. baumannii***	***S. aureus***	***S. epidermidis***
EOFv	8	10	14	-	26	20	16
RA_{30}	10	12	14	-	20	29	15
NET_{30}	NT	NT	NT	NT	15	17	NT
LVX_5	18	NT	NT	18	-	22	NT

Table 5. Minimum inhibitory concentration (MIC) of *F. vulgare* EO [(-): Inhibition; (+): Growth; C: Control].

Germs	**1/100(v/v)**	**1/250(v/v)**	**1/500(v/v)**	**1/1000(v/v)**	**1/2000(v/v)**	**1/3000(v/v)**	**1/5000(v/v)**	**C**
E.coli	+	+	+	+	+	+	+	+
S. aureus	-	-	-	-	+	+	+	+
S. epidermidis	-	-	-	-	+	+	+	+
E. cloacae	-	+	+	+	+	+	+	+
K. pneumoniae	-	-	+	+	+	+	+	+
A. baumannii	-	-	-	-	-	+	+	+

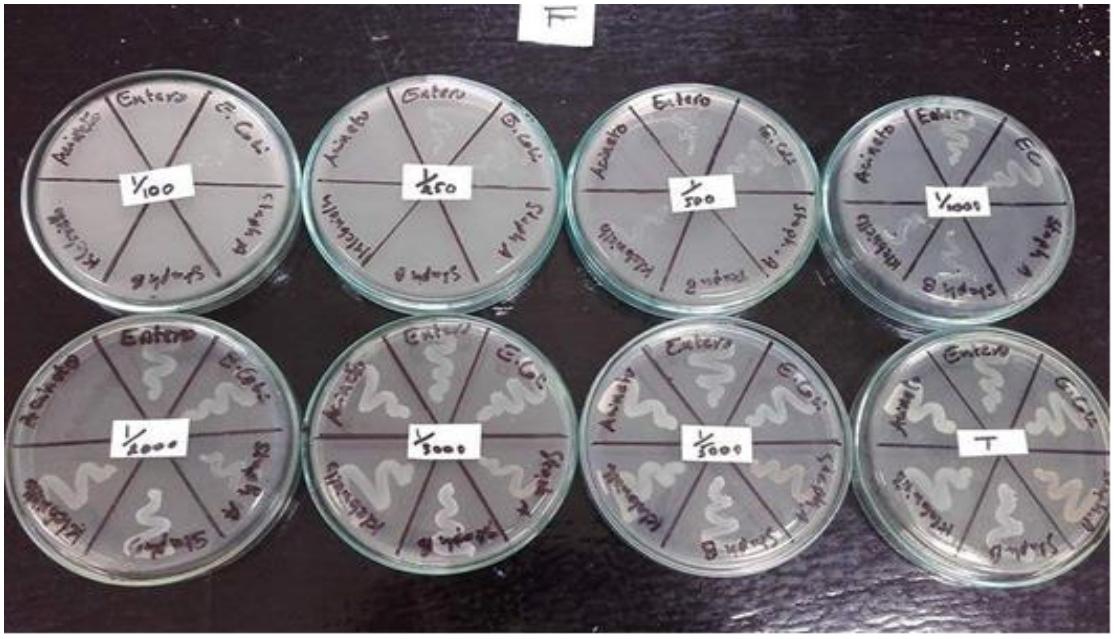

Fig. 2. Minimum inhibitory concentration (MIC) of *F. vulgare* EO against pathogenic strains.

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Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is the lowest concentration of the fennel EO to which 99.99% of bacteria are killed, after 24 h incubation at 37 °C. In this assay, it has been sought to determine the bactericidal effect of our plant (Table 6).

According to the MBC values, the best activity was observed against *A. baumannii* and *S. aureus* with a MBC value of 1/250 (v/v) followed by *S. epidermidis* and *K. pneumonia* with a bactericidal concentration of 1/100 (v/v) for both of them, while *E. cloacae* were the most resistant bacterium to all examined EO concentrations.

Table. 6. The Minimal bactericidal concentration (MBC) of the *F. vulgare* EO [(-): Inhibition; (+): Growth].

Bacteria	1/100(v/v)	1/250(v/v)	1/500(v/v)	1/1000(v/v)	1/2000(v/v)
E. cloacae	+	+	+	+	+
A. baumannii	-	-	+	+	+
S. aureus	-	-	+	+	+
S. epidermidis	-	+	+	+	+
K. pneumonia	-	+	+	+	+

DISCUSSION

The *Foeniculum vulgare* essential oil (fennel EO) yield was found to be 2.82%. The obtained average value is interesting and can be profitable to the industrial scale. The yields of unripe and ripe *F.v.* subsp. *piperitum* seeds in Turkey were 6.01 % and 4.41 %, respectively (Özcan *et al*. 2006), which are higher than the one we have recorded. However, Ghouati *et al.* (2014) and Roby *et al*. (2013) have shown that *F. vulgare* seed in Morocco and Egypt are characterized by a low EO yield (2.22 % and 1.95 % respectively). The results obtained by Khammassi *et al.* (2018) on the species from several regions of Tunisia also revealed a variation in yield depending on the origin of the plant. Thus, the obtained values of *F. vulgare* seed yields vary from 1.2% to 5.06%. Our results were among the lowest and highest reported values. Other studies carried out by Diao *et al*. (2014), on the *F. vulgare* seeds obtained as a commercial product in October, revealed a yield of 1.74%, similar to those reported by Roby *et al*. (2013) who also managed to extract 1.95% from the same species. Many factors influence the yield, the content and the chemical composition of essential oils, such as, the species, geographical origin, environmental conditions (*Faudale et al*. 2008; Ghasemian *et al*. 2019), the technique of extraction, drying, the period and the middle of crop, cropping practices as well as the age of the plant material. (Aberchane *et al*. 2001, Bourkhiss *et al*. 2011).

The results of the chemical analysis revealed that the five major constituents are trans-anethole (44.376%), 1-butanone, 2-chloro-3-methyl-1- [4- (1-methylethyl) phenyl]- (19.411%), D-limonene (8.287%) and estragole (5.092%) (Fig. 1). The EO of the same plant, i.e., *F. vulgare* of Iranian origin (Tehran) gave a majority composition of 99.65% chemical constituent, including trans-anethole (88.61%), estragole (3.02%), anethole (88.61%), estragole (3.02 %), fenchone (2.28%), 1-8-cineole (2.15%), p-anisaldehyde (1.34%), limonene (0.55 %) and linalool (0.51 %) (Salami *et al*. 2016). Cerpa Chavez and his colleagues reported that trans-anethole is the main component of the fennel EO, followed by fenchone, pinene, methyl-chavreol, phellandrene, and d-limonene. (Cerpa Chávez *et al*. 2007).

The extracted fennel EO harvested from Sétif in Algeria is mainly composed of trans-anethole 72.86%, fenchone 12.93 %, limonene 6.37 % and estragole 3.41 % (Boubiri *et al*. 2010). Mimica-Dukic *et al*. (2003) also reported Trans-anethole (72.7%-74.18%), fenchone (11.32%-16.35%), estragole (3.78%-5.29%), α-pinene (2.12%-2.77%) and limonene (1.80%-2.53%) as the important compounds identified in the fennel extracted oil. Yamini *et al*. (2002) found that chemical analysis of fennel seed EO of Iranian origin extracted by hydro-distillation, has mainly E-anethole (69.41%), fenchone (11%), limonene (10%) and 4.45% of estragole. In addition, Moura *et al*. (2005) have shown that the main constituents of fennel seeds EO from Brazil obtained by hydro-distillation are trans-anethole (74.2%) and fenchone with 15%. On the other hand, in Italy (Sicilian), Napoli and his collaborators found two dominant compounds, estragole (70.0 to 81.2%) and fenchone (9.7 to 18.7%) (Napoli *et al.* 2010). Furthermore, Ruberto *et al*. (2000) noted that estragole (55.3%) is the majority compound with α-pinene (11.3%) and α-phellandrene (9.1%). Moreover, the fennel EO is characterized by a

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
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very interesting chemical polymorphism that has been reported by Wodnicka *et al*. (2019). Indeed, they showed that the *F. vulgare* seeds grown in Poland contained 4.14% EO with trans-anethole (69.95%) as the main component, while Egyptian plant material is characterized by the low EO content (1.32%), with a predominant share of estragole (87.49%).

The profile found by Lahhit *et al*. (2011) is distinctly different from those found in the literature. The predominant compounds of the oil they worked on contained limonene (20.8%) and β-pinene (17.8%). In addition, Upadhyay (2015) isolated 36 components of fennel EO from Gorakhpur, Uttar Pradesh, India. The main constituents were 9-octadecenoic acid (18.56%), o-benzenedicarboxylic acid (14.47%), 1,3,3-trimethyl-2-vinyl-1-cyclohexene (10.77%), 1H-benzocycloheptene (10.71 %), 8Z)-14-methyl-8-hexadecenal (7.75%), 2-methyl-3-oxoestran-17-yl acetate (5.46%), pentad ecanecarboxylic acid (4.25%).

These quantitative and qualitative modifications in the chemical composition of the EO observed in different countries could be attributed to several parameters such as: agro-climatic factors (Stefanini *et al*. 2006), geography (Diaz-Maroto *et al*. 2006; Raal *et al*. 2011), the cultivated variety, the maturity stage of fennel seeds and the extraction method (Hammouda *et al*. 2013).

According to the results, the growth inhibition was observed in all of the pathogen strains, with the exception of the *P. aeruginosa* and *E. coli*, which were more resistant than the others. By contrast, *A. baumannii* and *S. aureus* were more sensitive to the fennel EO. In addition, the results of antimicrobial activity of the three standard antibiotics examined indicated that *P. aeruginosa* was the most resistant strain followed by *E. coli*, while *S. aureus* was the most sensitive to Rifampicin than to the other two antibiotics. Moreover, Levofloxacin was the most effective antibiotic and showed potent antibacterial activity against *P. aeruginosa* and *E. coli*. However, the fennel EO showed more activity than the standard antibiotics against *A. baumannii*. In general, essential oils have a very large spectrum of antimicrobial activity, against all types of microorganisms, not only bacteria, but also fungi and even viruses (Thormar 2011). While the activity spectrum of antibiotics is generally limited to a type of microorganism, for example bacteria, and even against a very specific family or species (Walsh 2003).

Ruberto *et al*. (2000), reported that the antibacterial activity of fennel EO against *E. coli* and *S. aureus*, exhibited the inhibition zones of 7.25 mm and 16.5 mm, respectively. Although Dinesh *et al*. (2014), reported that the inhibition zone caused by fennel EO against *E. coli* was 28 mm, however, our data revealed lesser antimicrobial activity against *E. coli* (8 mm). Our result was opposed to that recorded by Miguel *et al*. (2010) who reported a very low antimicrobial activity of the fennel EO.

In the present study, the inhibitory effects of the fennel EO were found to be varied against the examined bacteria, which might be due to the differences in the biological properties of the main compounds in the essential oils. Our findings are in agreement with those reported by Ghouati *et al*. (2014) who mentioned that MIC of *F. vulgare* seed oil against *S. aureus* was 1/1000(v/v).

Jazani *et al*. (2009) studied the antibacterial effects of fennel EO on *A. baumannii* strains which cause nosocomial infection. The results of this study showed that the fennel EO possessed antibacterial activity against all isolates of *A. baumannii*. In other studies, a minimum concentration of fennel EO that inhibits the growth of *S. aureus* and *E. coli* was ≤ 0.025%. (Akrayi 2012). Abdurahim *et al*. (2017) also reported that *S. aureus* and *E. coli* were indicated admirable activity.

The results of our study on MBC of *F. vulgare* seed OE exhibited bactericidal effects on the four strains examined with exception of *E. cloacae*, which presented a bacteriostatic effect. In this study, the antibacterial activity could be related to the major compounds like trans-anethole, estragole and fenchone, which are all reported for their antibacterial potency (Cowan. 1999), while the other constituents could act synergistically. Similarly, the results of Bakkali *et al*. (2008) and Kalemba & Kunicka (2003) showed that the antibacterial activity would result from the positive implication of all the EO constituents. On the other hand, Dadalioglu & Evrendilek (2004) demonstrated that the antibacterial activity of the fennel seed EO is due to the major component, i.e., anethole, and these observations have been confirmed by Ağaoğlu *et al*. (2007), Gulfraz *et al*. (2008) and Abdulrahman *et al*. (2010).

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DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research

CONCLUSION

The *Foeniculum vulgare* essential oil yield was found to be 2.82 %. The obtained average value is interesting and can be profitable to the industrial scale. The study of the chromatographic profile of our fennel seed extracts, using CG/MS has allowed the identification of 25 compounds including trans-anethole (44.376%), 1-Butanone, 2-chloro-3-methyl-1-[4-(1-methylethyl) phenyl]- (19.411%), L-Fenchone (11.728%), D-limonene (8.287%) and the estragole (5.092%). The antibacterial activity of fennel EO on the examined pathogens was very effective. The inhibition of growth was observed in the majority of the examined strains, with the exception of the *P. aeroginosae* and *E. coli*, which were more resistant than the others. By contrast, *A. baumannii* and *S. aureus* were more sensitive to the fennel OE. On the one hand, this confirms the effectiveness of the fennel seeds in the traditional pharmacopoeia and on the other hand, opens the prospect of the developing new antibacterial products. The essential oils of the Moroccan species may also have a word in the field of reduced human infection problems.

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. Abdelkader Zarrouk, from Mohammed V University, Rabat, Morocco for the support of this research.

Conflicts of Interest

There are no conflicts of interest**.**

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DOI: 10.22124/cjes.2020.4276
Received: Jan. 28. 2020 Accepted: Jun 09. 2020
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Article type: Research

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DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research

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Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276

Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research

ترکیب شیمیایی و ارزیابی فعالیت ضد باکتریایی گیاه رازیانه *(Foeniculum vulgare)* در مقابل برخی از سویه‌های باکتریایی بیماری زا

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(تاریخ دریافت: ۹۸/۱۱/۰۸ تاریخ پذیرش: ۹۹/۰۳/۲۰)

چکیده

هدف از این کار بررسی فعالیت ضد باکتریایی در شرایط *in vitro* روغن استخراج شده از بذرهای خشک گیاه *Foeniculum vulgare* Mill، جمع‌آوری شده از مکنس، مراکش است که در برابر هفت باکتری بیماری‌زا آزمایش شده است : *Escherichia coli*، *Klebsiella pneumoniae*، *Enterobacter cloacae*، *Pseudomonas aeruginosa*، *Acinetobacter baumannii*، *Staphylococcus epidermidis* و *Staphylococcus aureus* استخراج روغن ضروری از گیاه *Foeniculum vulgare* Mill با استفاده از روش تقطیر آبی در دستگاه نوع Clevenger انجام شد. عملکرد نزدیک به ۲/۸۲٪ بود. شناسایی ترکیب شیمیایی روغن رازیانه توسط کروماتوگرافی گازی همراه با طیف سنجی جرمی (GC / MS)، ۲۵ ماده متشکله را نشان داده است. ۹۷/۵۵٪ کل عناصر تشکیل دهنده روغن موجود را تشکیل می‌دهند. ترکیب اصلی ترانس آنتول با (۳۷/۴۴۶٪) بود. نتیجه این مطالعه نشان داد که روغن اسانس *Foeniculum vulgare* نسبت به سه آنتی بیوتیک استاندارد ، فعالیت مهاری قابل توجهی در برابر بیشتر ریزموجودات های آزمایش شده ، به خصوص *Acinetobacter baumannii*، بجز *Pseudomonas aeruginosa* و *Escherichia coli* دارد. روغن عصاره رازیانه دارای یک ضد میکروب قوی در برابر *Acinetobacter baumannii* (با منطقه مهار رشد ۲۶ میلی متر) در مقایسه با آنتی‌بیوتیک‌های استاندارد مورد مطالعه، با حداقل غلظت مهاری (MIC) ۱/۲۰۰۰ (حجم\ حجم) و استافیلوکوکوس اورئوس (منطقه مهار رشد ۲۰ میلی متر و با MIC ۱/۱۰۰۰۰ حجم\ حجم) بود ، این نتایج نشان می دهد که اسانس مورد آزمایش یک منبع بالقوه از مواد ضد باکتری طبیعی است که می‌توان آن را در برابر سویه های بیماری زا استفاده کرد.

*مولف مسئول

Bibliographic information of this paper for citing:

Barrahi, M, Esmail, A, Elhartiti, H, Chahboun, N, Benali, A, Amiyare, R, Lakhrissi, B, Rhaiem, N, Zarrouk, A, Ouhssine, M 2020, Chemical composition and evaluation of antibacterial activity of fennel (*Foeniculum vulgare* Mill) seed essential oil against some pathogenic bacterial strains. Caspian Journal of Environmental Sciences, 18: 295-307

Caspian J. Environ. Sci. Vol. 18 No. 4 pp. 295~307
DOI: 10.22124/cjes.2020.4276
Received: Jan. 28. 2020 Accepted: Jun 09. 2020
Article type: Research