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Research Article

Biological activity of essential oil from *Foeniculum vulgare*

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Foeniculum vulgare Mill. is a medicinal plant, used as a flavouring agent. The essential oil from *F. vulgare* has potential antimicrobial and insecticidal effects, and can be used in food industry in order to protect the food resources and food products against microbial and pest's contamination. The aim of the research was to characterize the volatile components of *F. vulgare* essential oil by Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography (GC-FID) and to observe the antimicrobial activity by disk diffusion method and in vapour phase. Also, insecticidal activity of the vapour phase of the essential oil of *F. vulgare* was detected. We found that major components of the essential oil from *F. vulgare* were *trans*-anethole (73.6%), fenchone (6.0%), and limonene (5.7%). Antimicrobial activity on gram-positive, gram-negative, and yeasts was weak in liquid phase, but vapour phase showed stronger activity against *B. subtilis* at the concentration 250 $\mu L.L^{-1}$ (98.65% of bacterial growth inhibition). Vapour phase of essential oil was effective against insects, where 25% concentration had 80% lethality.

Keywords: *Foeniculum vulgare*, antimicrobial activity, essential oil, vapour phase

1 Introduction

Foeniculum vulgare Mill., commonly called the fennel, is a perennial plant that belongs to carrot family Apiaceae. The medicinal plant with yellow flowers, aromatic seeds and white fruits is widely used as a flavouring agent of foods and beverages for its characteristic aroma (Rather et al., 2016). Fennel is used in traditional medicine for many years due to beneficial antioxidant, anti-inflammatory and analgesic effects (Choi & Hwang, 2004). The fennel is full of phytochemicals, especially volatile compounds extracted to essential oils (EOs) (Badgujar et al., 2014). *Foeniculum vulgare* EOs have hepatoprotective and antidiabetic properties on *in vivo* models (Abou et al., 2011; Özbek et al., 2003). With the high concentration of *trans*-anethole, EOs from *F. vulgare* possess a strong antioxidant activity (Shahat et al., 2011). The antimicrobial activity against gram-positive, gram-negative, and fungi was observed, thus volatile compounds of fennel EO can be used in food preservation (Diao et al., 2014; Mimica-Dukić et al., 2003). Insecticidal activity against *Trogoderma granarium* (Ghanem et al., 2014), *Brevicoryne brassiceae* (Lucca et al., 2015), *Acyrthosiphon pisum*, and *Myzus persicae* (Digilio et al., 2008) was also observed.

Nowadays, the use of natural substances over the artificial ones has been increasing in order to preserve food resources against pathogens, both microscopic ones and pests. Essential oils have various positive biological effects that can be applicable in food preservation. Due to the volatility of the substances, essential oils can be applied without the direct contact with a food resource, thus addition of natural food preserver will not change the chemical or sensory properties of the foods.

The aim of this study was to characterize the essential oil from *F. vulgare* Mill var. dulce from a Slovak company to obtain the chemical composition of the EO from

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F. vulgare and to observe the activity of the gas phase against pathogenic microorganisms on the carrot used as a food model. Also, the insecticidal activity against the *Pyrrhocoris apterus* in the gas phase was detected.

2 Material and methods

2.1 Essential oil

EO prepared from *Foeniculum vulgare* Mill var. dulce was purchased from Hanus, s.r.o. (Nitra, Slovakia) and was extracted by steam distillation of dried fruits. It was stored in the dark at 4 °C throughout the analyses.

2.2 Tested microorganisms

Microorganisms (*Bacillus subtilis* CCM 1999, *Pseudomonas aeruginosa* CCM 3955, *Yersinia enterocolitica* CCM 7204, *Staphylococcus aureus* subsp. *aureus* CCM 8223, *Enterococcus faecalis* CCM 4224, *Salmonella enteritidis* subsp. *enteritidis* CCM 4420, *Candida krusei* CCM 8271, *Candida albicans* CCM 8261, *Candida tropicalis* CCM 8223, and *Candida glabrata* CCM 8270) were obtained from the Czech collection of microorganisms. The biofilm-forming bacteria *Bacillus subtilis* and *Stenotrophomonas maltophilia* were obtained from the dairy industry and identified with 16S rRNA sequencing and MALDI-TOF MS Biotyper.

2.3 Chemical characterization of essential oil by gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC-FID)

GC/MS analysis of the EO sample was performed using Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to quadrupole mass spectrometer 5975B (Agilent Technologies, Santa Clara, CA, USA). A HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used. The temperature program was set from 60 °C to 150 °C (increasing rate 3 °C.min^{-1}) and 150 °C to 280 °C (increasing rate 5 °C.min^{-1}). The total running time was 60 min. Helium 5.0 was used as the carrier gas with the flow rate of 1 mL.min^{-1}. The injection volume was 1 µL (the EO sample was diluted in pentane), while the split/splitless injector temperature was set at 280 °C. The sample was injected in the split mode with the split ratio at 40.8 : 1. Electron-impact mass spectrometric data (EI-MS; 70 eV) were acquired in scan mode over the m/z range 35–550. MS ion source temperatures was 230 °C and MS quadrupole temperature was 150 °C. Acquisition of data started after solvent delay time of 3 min. GC-FID analyses were performed on Agilent 6890N gas chromatograph coupled to FID detector. Column (HP-5MS) and chromatographic conditions were the same as for GC/MS. The temperature of the FID detector was set at 300 °C.

The volatile constituents of samples were identified according to their retention indices (Adams, 2007) and they were compared with the reference spectra (Wiley and NIST databases). The retention indices were experimentally determined by a standard method described by (van Den Dool & Dec Kratz, 1963) and included retention times of *n*-alkanes (C6-C34), injected under the same chromatographic conditions. The percentages of the identified compounds (amounts higher than 0.1%) were derived from the GC peak areas.

2.4 Disk diffusion method

The antimicrobial activity of *F. vulgare* EO was determined using the disk diffusion method. Cultivation of microorganisms was performed aerobically for 24 h on Tryptone soy agar (TSA). The bacteria were incubated at 37 °C and the yeasts at 25 °C. The inoculum was prepared to an optical density of 0.5 McFarland (1.5 × 10^8 CFU. mL^{-1}). 100 µL of inoculum was spread on Petri dishes (PD) with Mueller Hinton agar. A sterile 6 mm diameter paper blank disks were placed on the PD and, subsequently, 10 µL of 100% *F. vulgare* EO was applied. The prepared PDs was incubated aerobically for 24 h, the bacteria at 37 °C and the yeast at 25 °C. The criteria for detecting inhibitory activity were: inhibition zone diameter below 5 mm – very weak inhibitory activity, above 5 mm – weak inhibitory activity, above 10 mm – medium inhibition and above 15 mm – very strong inhibitory activity. Each test was repeated three times. The measurement was performed in triplicate, the mean and the standard deviation was calculated.

2.5 Antimicrobial activity of the vapour phase of the Essential oil

The *in situ* antimicrobial effect of *F. vulgare* EO against biofilm-forming microorganisms *B. subtilis* and *S. maltophilia* was evaluated on carrots. The carrots were cut into 5 mm slices, washed with distilled water, and left to dry for 15 minutes at room temperature. A thin layer of Mueller Hinton agar (MHA) was poured into 60 mm diameter PDs and their lids. The individual carrot slices were placed on solidified MHA. A bacterial inoculum with an optical density 0.5 McFarland (1.5 × 10^8 CFU.mL^{-1}) was prepared. The inoculum was applied by three stabs into the carrot slice. *F. vulgare* essential oil was diluted in 100% ethyl acetate to concentrations 500, 250, 125, and 62.5 µL.L^{-1}. A circle of sterile 55 mm diameter filter paper was placed on the solidified MHA in the lid and 100 µL of the appropriate concentration of essential oil was applied on the filter paper. 100 µL of ethyl acetate was used as a negative control. The ethyl acetate was evaporated during the 1 minute and, subsequently, the PDs were hermetically sealed. The samples were incubated at 37 °C for 7 days.

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The antimicrobial effect of *F. vulgare* EO was evaluated using a stereological method. The bulk density (Vv) of the bacterial colonies was estimated using ImageJ software. The *Vv* was calculated using the formula *Vv* (%) = *P*/*p* × 100, where *P* represents the stereological grid points where the bacterial colonies were grown, and p represents all the stereological grid points on the substrate. The volume density of the colonies was the percentage inhibition of *F. vulgare* EO calculated using the formula BGI = [(*C* – *T*)/*C*] × 100, where *C* is the bulk density of the bacterial colonies in the control group and *T* is the bulk density of the colonies in the treated samples.

2.6 Vapour phase insecticidal activity

The insecticidal activity of *F. vulgare* EO was tested using *Pyrrhocoris apterus*. The EO was diluted in 0.1% polysorbate solution. The concentrations of 25, 12.5, and 6.25% were tested. 0.1% polysorbate was used as a negative control. 30 individuals of *P. apterus* were placed in 90 mm PD with vents. A circle of sterile filter paper was placed into the PD lid. 100 µL of the appropriate concentration of EO was applied to the filter paper and the plates were sealed with parafilm. *P. apterus* were exposed to EO vapours for 24 h at room temperature. After exposure, live and dead subjects were counted, and the percentage of insecticidal activity was calculated.

3 Results and discussion

3.1 Chemical composition of the EO

The GC/MS and GC-FID analyses showed (Table 1) that the major component of *F. vulgare* EO was *trans*-anethole with 73.6%, fenchone with 6.0%, and limonene with 5.7%. Trans-anethole is the major component of the EOs from *F. vulgare*, and fenchone, estragole, and limonene have been also reported as main components of essential oils derived from the seeds of *F. vulgare* (Belabdelli et al., 2020; Diao et al., 2014; El-Nasr et al., 2013). The content of the individual components of the essential oil can be influenced by geographical and environmental factors (Piccaglia & Marotti, 2001) Also, the extraction method can affect the chemical composition of the essential oil (Bagherifard et al., 2014).

Table 1 Chemical composition of *F. vulgare* EO

No	RI	Identified compounds	(%)
1	938	α-pinene	4.4
2	980	β-pinene	0.4
3	992	β-myrcene	0.5
4	1004	α-phellandrene	1.7
5	1023	*p*-cymene	0.3
6	1028	α-limonene	5.7
7	1030	β-phellandrene	1.7
8	1060	γ-terpinene	0.2
9	1085	fenchone	6.0
10	1195	methyl chavicol	4.2
11	1252	*p*-anisaldehyde	0.6
12	1284	*trans*-anethole	73.6
Total			99.3

RI – values of retention indices on HP-5MS column; compounds identified in amounts higher than 0.1%

3.2 Antimicrobial activity

The results of the disk diffusion method are shown in Table 2. Only very weak to weak inhibitory activity was observed for all tested microorganisms. Among the all tested microorganisms, the inhibition of *B. subtilis* was the most pronounced one.

Garzoli et al. (2018) reported that due to the increased content of estragole, limonene and fenchone, the essential oil of *F. vulgare* has an inhibitory effect on the growth of the genus *Candida*. In our study, despite the presence of these components in the tested essential oil, we did not observe any significant antimicrobial activity against the genus *Candida*. Alzoreky and Nakahara (2003) found in their work that the extracts obtained from dried seeds of *F. vulgare* show weak antimicrobial activity. Differences in the composition and percentage of active ingredients in EO (Bozin et al., 2006), species, subspecies or diversity of plants, geographical locations, methods of collection, drying and extraction in the production of essential oils can greatly affect the antimicrobial

Table 2 Disk diffusion method in the *F. vulgare* EO against G+, G- bacteria, and yeasts (inhibition zones in mm)

Microorganism	Zone inhibition
Bacillus subtilis	7.78 ±1.39
Pseudomonas aeruginosa	3.00 ±0.71
Enterococcus faecalis	4.67 ±0.87
Yersinia enterocolitica	4.67 ±0.87
Salmonella enteritidis	3.33 ±0.71
Staphylococcus aureus	3.56 ±1.01
Candida glabrata	5.00 ±0.87
Candida krusei	5.33 ±0.87
Candida albicans	5.89 ±1.27
Candida tropicalis	5.22 ±1.48
***Stenotrophomonas maltophilia* biofilm**	3.56 ±1.01
***Bacillus subtilis* biofilm**	7.89 ±0.93

Table 3 Inhibitory activity of vapour phase of *F. vulgare* EO on carrot samples

Bacterial growth inhibition (%)				
Microorganisms	**concentration (µL.L^{-1})**			
	62.5	**125**	**250**	**500**
Stenotrophomonas maltophilia	2.15	23.98	5.21	14.44
Bacillus subtilis	0.29	1.48	98.65	100.00

Table 4 insecticidal activity of vapour phase of *F. vulgare* EO against *P. apterus*

Concentration (%)	**Number of living individuals**	**Number of dead individuals**	**Insecticidal activity (%)**
25	6	24	80
12.5	12	18	60
6.25	18	12	40
Control group	30	0	0

properties of the essential oil (Burt, 2004; Sarac & Ugur, 2008).

3.3 Antimicrobial activity in vapour phase

The results of the antibacterial activity of the gas phase of *F. vulgare* essential oil on carrots are summarized in Table 3. *S. maltophilia* was maximally inhibited at 125 µL.L^{-1} by 23.98%. The lowest inhibition was found at the concentration of 62.5 µL.L^{-1}. *B. subtilis* was inhibited by the concentration of 250 µL.L^{-1} by 98.65% and at 500 µL.L^{-1} the inhibition was at 100%. As with *S. maltophilia*, the lowest inhibition at 62.5 µL.L^{-1} was only 0.29%.

The vapour phase method makes it possible to monitor the antimicrobial activity of exclusively volatile components of the essential oil. The vapour state can increase the antimicrobial effect at lower concentrations compared to contact antimicrobial activity of the liquid EO (Ács et al., 2018). Dorman and Deans (2000), Garzoli et al. (2021), and Nedorostova et al. (2009) confirmed the higher effectiveness of antimicrobial effects of the vapour phase than liquid phase of essential oils.

3.4 Insecticidal activity

The results of the insecticidal activity of the vapour phase of *F. vulgare* EO against *P. apterus* are shown in Table 4. All concentrations tested showed insecticidal activity. It was most pronounced at a concentration of 25% where we recorded the killing of 80% of individuals. At the concentration of 12.5%, we observed the killing of 60% of individuals, and at the concentration of 6.25%, 40% of individuals were killed.

Effective insecticidal activity against three types of insects was observed by Pavela et al. (2016). The insecticidal effect of the essential oil depends mainly on the substances that the essential oil contains (Bakkali et al., 2008). *F. vulgare* EOs can be used as insect control agents and can be useful in managing field plant populations (Kim et al., 2002).

4 Conclusions

The essential oil from *F. vulgare* showed good antimicrobial and insecticidal activity due to its volatile compounds. The major component was *trans*-anethole in quantity 73.6%. Fenchone, α-limonene, methyl chavicol, and α-pinene were also present. The vapour phase effectively inhibited the growth of the *B. subtilis*. Also, insecticide activity was observed against *P. apterus*. EO from *F. vulgare* has potential in use as natural supplement in food industry, due to ability to inhibit pathogenic microorganisms. Also, EOs can be potentially used on food resources and industrial crops, due to insecticide activity. Use of volatile compounds present in essential oils can be also advantage, because the sensory properties of the product will not be changed.

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