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Chemical composition, antifungal and antibacterial potential of fennel (*Foeniculum vulgare)* and cumin (*Carum carvi)* essential oils (Apiaceae)

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Abstract

The aim of this study was to evaluate and compare antimicrobial activity of the essential oils of two Apiaceae species, cumin (*Carum carvi*) and fennel (*Foeniculum vulgare*) . The essential oils from *Carum carvi* and *Foeniculum vulgare* fruits were isolated by hydrodistillation and analysed by gas chromatography with mass spectrometry (GC-MS). Antimicrobial activity of essential oils was tested against standardised bacterial and fungi cultures: *Staphylococcus aureus* ATCC 25923, *Esherichia coli* ATCC 25922, *Candida albicans* ATCC 24433, *Enterococcus faecalis* ATCC 25929, using the agar diffusion method with wells.

Gas chromatography with mass spectrometry (GC-MS) revealed that the main components of the essential oil of cumin were limonene (29.85%), p-cymene (25.60%), α pinene (13.25%) and eucalyptol (16.98%). The major constituents of fennel were trans-anethole (63.16%), l-fenchone (15.53%), estragole (6.43%), limonene (4.69%) and α pinene (4.33%). Minimum inhibitory concentration (MIC) for essential oils has been determined by the broth dilution method and valued in the range of 50 μL/ml - 100 μL/ml, depends on essential oil and bacteria tested, and for *C. albicans*. Moreover, the combination of essential oils and lactic acid confirmed synergistic and additive activities against the pathogens. Both essential oils, cumin on the first place, showed an inhibitory effect against most of tested bacteria and fungi. The Gram positive bacteria *E. faecalis* was resistant to both essential oils, as well as Gram negative bacteria *E. coli* that was resistant to the fennel essential oil activity. These results indicate that cumin essential oil have significant antimicrobial potential and it can be used in antimicrobial therapy as natural antimicrobial agent, or in combination with the other antimicrobial agents, which could be the subject of the further investigations.

Key words: cumin; fennel; essential oils; antimicrobial activity

Antibacterial resistance becomes global problem and has forced us to search for new and efficient antimicrobial agents. Therefore, there is an ongoing search for new natural substances which have antibacterial effects, the most important ones being plant secondary metabolites, alongside their essential oils. Essential oils show significant antimicrobial activity that is documented by a number of studies and has been used in folk medicine for centuries. A large number of aromatic drugs, which have been widely used in the traditional medicine, but whose application in the official therapy of various diseases has been on the rise as well, originate from the types of families of Apiaceae. These drugs (*Coriandrum sativum, Foeniculum vulgare, Carum carvi*, etc.) are characterized by a distinct production of the secondary metabolites (essential oils), that is why some of them such as cumin and fennel have been used since the ancient times as spices in the daily diet (Bakkali et al., 2008). Essen-

tial oils have a wide spectrum of bioactivity, owing to the presence of several active ingredients (e.g., terpenes and phenol-derived aromatic and aliphatic components) that have various modes of action. The dominant components of the essential oils are compounds from the class of terpenoids, namely mono- (C-10) and sesquiterpenes (C-15). In the volatile fractions there are mostly lower monoterpenes and simpler sesquiterpenes, while the polyoxidised sesquiterpenes and diterpenes can be found in the less easily volatile fractions and resins (Bozin et al., 2006).

Ever since ancient times, essential oils of Apiaceae family are used for its pharmacological effects as a stomachic (enhances the appetite and simulates the gastric secretion), cholagog (enhances the bile secretion) and carminative (when experiencing tympanitis and swelling due to spasmodic activity of various components), while in the food industry they are used as spice, korigens and food preservative, and they are also used in the cosmetic and perfume industry (Lo Cantore et al., 2004). These essential oils also demonstrates a significant antimicrobial and antifungal effect (Ruberto et al., 2000). Due to the variety of chemical components of the essential oils, it is presumed that their antimicrobial activity is not based on one specific mechanism of operation (Pai et al., 2010; Sikkema et al., 1994). The lipophilic character of the components of the essential oil (EO) suggests interaction with bacterial membranes as the main mechanism of the antibacterial action, disturbing the structure and rendering it more permeable leading into death of bacterial cell (Burt, 2004).

Such strong antimicrobial activity of essential oils on pathogenic bacteria is based on a high level of phenolic component. Dorman and Deans (2000) noticed that this mechanism of antibacterial activity is based on lipophilic properties of constituents of essential oils and their functional groups. Due to the variety of chemical components of these essential oils, it is presumed that their antimicrobial activity is not based on one specific mechanism of action and that these lipophile oils has multiple target places on cytoplasmic membrane change its permeability (Gachkar et al., 2007; Iacobellis et al., 2005). There is no scientific evidence of resistance on essential oils yet, that is explained by great complexity of their structure and the fact that essential oils are active for several target places at the same time.

Phenols, phenolic glycosides and volatile aroma compounds such as trans-anethole, estragole and fenchone have been reported as the major phytoconstituents of the fennel EO (Diao et al., 2014). There are many *in vitro* and *in vivo* studies that demonstrate antifungal, antibacterial and antioxidant activities of fennel EO (Singh et al., 2006b; Roby et al., 2013).The EO of cumin also demonstrates a significant antimicrobial, or rather, the antifungal effect (Eikani et al., 2007). It is very important to notify that cumin and fennel essential oils are regarded generally as safe (GRAS 2340 and GRAS 2343) under the regulatory system and no adverse effects have been reported from the use of these essential oils to date (Parthasarathy and Zachariah, 2008).

Considering the above, the aim of the present study is to investigate the potential antimicrobial activity of these two essential oils, commonly used in folk medicine, and their possibility of use as a natural antibiotic.

MATERIAL AND METHODS

Plant material

Fruits of the cumin and fennel were obtained at the Institute for the study of medicinal herbs "Dr. Josif Pančić" in Belgrade in 2010. The plant material was kept in the double paper bags in a dark and dry place until hydrodestilation. Immediately prior to the hydrodestilation, the fruit of cumin (*Carvi Fructus*) and fennel (*Foeniculum Fructus*) were chopped to the size of 0.75 mm (Pharmacopoea Jugoslavica, 1984).

Hydrodestillation (HD)

The essential oils were isolated through the process of hydrodestilation as per pH. Eur. IV, with *n*-hexane as the collective dissolver. The amount of 500 ml of distilled water was poured over the plant material (25 g). The obtained essential oils was initially dried by means of anhydrite Na_2SO_4 for 24 h, and then the drying process continued in the desiccator for another hour. The *n*-hexane was boiled in the rotational vacuum-boiler, and then the obtained EO was measured three times and its quantity expressed as per the mass of the dry plant material

(g/100 g). Dried oils were preserved in cuvettes with Teflon stopper at +4°C until they have been analysed.

Gas chromatography with mass spectrometry (GC-MS)

Gas-chromatographic analysis of essential oils was conducted in Hewlett Packard 5973-689 GC-MS system in EI mode on 70eV with spectrometric detection of masses (GC-MS-Gas Chromatography-Mass Spectroscopy). The initial temperature of capillary column HP 5MS (30 m x 0.25 mm; film thickness 0.25 μm) was 60°C. Using the heating speed of 3°C/min, it was heated to 280°C. Helium was a gas carrier for this purpose, and it had the flow of 1 ml/min. The amount of 1 μl of each investigated sample was injected into the GC column in proportion of 1:10.

Identification of components was based on calculated retention indexes (RI) (Van den Dool and Kratz, 1963) and mass spectra compared with standard substances and/or with NIS/NBS Wiley library of mass spectra, including literature data or data from a free database (http//www. flavornet. org/iowtv.pherobase.com) (Adams, 2007). Experimental values of retention indexes are defined using "calibrated Automated Mass Spectral Deconvolution and Identification System software'' (AMDIS ver.2.1., DTRA/NIST, 2002). Results are compared with retention indexes from literature data and via internet database available.

Microorganism cultures

For the purpose of *in vitro* testing of the antimicrobial activity of the cumin and fennel fruit essential oils, the following standardized bacterial cultures were used (ATCC – American Type Culture Collection): *Staphylococcus aureus* ATCC 25*923, Esherichia coli A*TCC 25922, *Candida albicans* ATCC 24433, *Enterococcus faecalis* ATCC 29212. These cultures of microorganism were deposited in the collection of the bacteria cultures of the Department of Microbiology at Faculty of Technology, Belgrade, and the bacteria cultures of Institute of Virology and Immunology, Torlak.

Determination of antimicrobial activity

Antibacterial activity was determined by disc diffusion and agar-well diffusion method. Agar-well diffusion method was employed for the determination of antimicrobial activity of the essential oils. Tubules with diameter of 6 mm were placed on Petri plates with prepared sterile Miller-Hinton TSA (tryptonesoy agar - Torlak) surface, and after impregnated with soft agar (0.60% of agar) inoculated with indicator pathogenic strain (0.2 ml of 24-hours broth culture for 6 ml of soft agar). After firming of agar, tubules are removed and each of formed wells was filled with 20 μl of investigated essential. Plates are incubated at 37°C during 24 hours. As a positive control of antibacterial activity standard antibiotic – clyndamicin (10 μg/ml) and antimycotic nystatine were taken (30 μg/ml).

Antimicrobial activity of essential oils is present all over the inhibition zone which is measured and expressed in mm.

Evaluation of Minimal inhibitory concentration (MIC) using the agar dilution method

For the purpose of determining the minimal inhibitory concentration (MIC) of essential oils, the dilution method was used and performed in Miler Hinton broth. Each test tube with 2.997 ml of the base was filled with 3 μl of essential oil. The concentrations of 10, 30, 50 and 100 μl/ml of EOs of cumin and fennel were used. 1% inoculum were used for inoculation of broth with diluted essential oils and control without oils. The test tubes were incubated on 37°C.

During certain time intervals, after 1, 3, 8, 16 and 24 h, the change of optical density (OD) was followed with a colorimeter (MA 9504. Metrix), using yellow filter (575 nm). Increasing of optical density (OD) indicates an increasing of microorganism biomass in a broth. MIC is defined as the first concentration of EO that there is no visible growth of bacteria.

RESULTS

Results of qualitative and quantitative analysis of the chemical composition of essential oils are shown in Table 1.

Via the GC-MS analysis of the tested cumin and fennel essential oils it was identified 99.19% of fennel EO and 98.59% of the cumin EO. The most dominant components for cumin EO were monoterpene hydrocarbons (α–pinene-13.25% and limonene -29.85%), aromatic monoterpene hydrocarbon (*p*-

Table 1. Chemical composition of cumin and fennel essential oils

Components	Cumin (%)	Fennel (%)
Monoterpen hydrocarbons	**53.01**	**27.77**
α–Pinene	13.25	4.33
α–Phellandrene	0.655	1.77
α–Terpinene	1.05	0.09
β–Pinene	1.1	0.55
Camphene	0.21	0.1
δ–Carene	0.5	-
γ–Terpinene	2.96	0.15
Limonene	29.85	4.69
Myrcene	1.93	0.51
Sabinene	1.23	0.05
Terpinolene	0.27	-
L-Fenchone	-	15.53
Aromatic monoterpene hydrocarbons	**25.60**	**1.21**
p-Cymene	25.60	0.38
p-Anisaldehyde	-	0.83
Oxygenated monoterpenes	**19.98**	**6.59**
4-(1-Methylethyl)-2-cyclohexen-1-one	0.11	-
6-Methyl-5-hepten-2-one	0.17	-
α–Terpineol	2.03	-
Camphor	0.27	0.16
Eucalyptol	16.98	-
Isopropenyltoluene	0.18	-
Linalol	0.24	-
2,5-Dimethylhexa-2,4-diene	-	-
6-Hepten-1-ol	-	-
Estragole	-	6.43
Fenilpropanoides	**0.00**	**63.36**
trans-Anethole	-	0.20
trans-Anethole	-	63.16
Sesquiterpene hydrocarbons	**0.00**	**0.26**
β-Caryophyllene	-	0.11
(-)-Zingiberene	-	0.11
GERMACRENE-D	-	0.04
Total:	**98.59**	**99.19**

cymene-25.6%) and oxidised monoterpene eucalyptol (16.98%). More than a half of the total composition of EO of fennel is made of dominant compound – fenilpropanoide – trans-anethole (63.13%). It is followed by l-fenchone (15.53%), α–pinene-(4.33) and limonene (4.69%) that belongs to monoterpene hydrocarbons, and estragole (6.43%) belonging to oxidized monoterpene.

The antimicrobial activities of the cumin and fennel EO against tested microorganisms and their antimicrobial potency were qualitatively and quantitatively assessed by presence or absence of inhibition zone, zone diameter and MIC value. The results are given in Table 2 and Figure 1, 2 and 3. The cumin EO showed antibacterial and antifungal activity against all tested microorganisms, except *E. faecalis*. The EO of fennel showed weaker antibacterial activity reacting only against *S. aureus* and *C. albicans.*

DISSCUSION

Twenty one compounds of cumin EO and nineteen compounds of the fennel EO were identified by GC-MS in the present study. The most dominant components of the cumin EO were p-cymene, α-pinene, eucalyptol and limonene, and they are in accordance with the previous study about chemical composition of this oils (Iacobellis et al., 2005; Diao et al., 2014; Eikani et al., 2007). Also, there are a number of studies that prove that these components are responsible for the aroma and biological effects of cumin oil, as well for its antimicrobial effects (Gachkar et al., 2007; Iacobellis et al., 2005; Lis-Balchin et al., 1998; Özcan and Erkmen, 2001). The result of chemical analyses of fennel EO showed that the most dominant compounds of the fennel oil are trans-anethole (63.13%) with well-known antimicrobial potential (Fujita et al., 2007; Lo Cantore e al., 2004), followed by estragole, limonene and fenchone.

Cumin EO was particularly effective against *Staphylococcus aureus* ATCC 25923*,* with a diameter of inhibition about 2 mm. On the other hand, Gram-negative bacteria *E. coli* was less sensitive to cumin EO with a diameter of growth inhibition around 1.5 mm. MIC values indicate that the EO of cumin was efficient against 2 of 3 tested bacteria and *C. albicans*. The MIC values

Table 2. Antimicrobial activities of the cumin and fennel essential oils shown as inhibition zone diameter

EO	Inhibition zone diameter, mm			
	S. aureus	*E. coli*	*C. albicans*	*E. faecalis*
Cumin	0.2	1.5-2	0.2	0
Fennel	0.1	-	0.1	0
Lactic acid	-	-	-	-
Nystatine (30 µl/ml)	-	-	4	-
Clindamycin (10 µl/ml)*	2	2	-	2.5

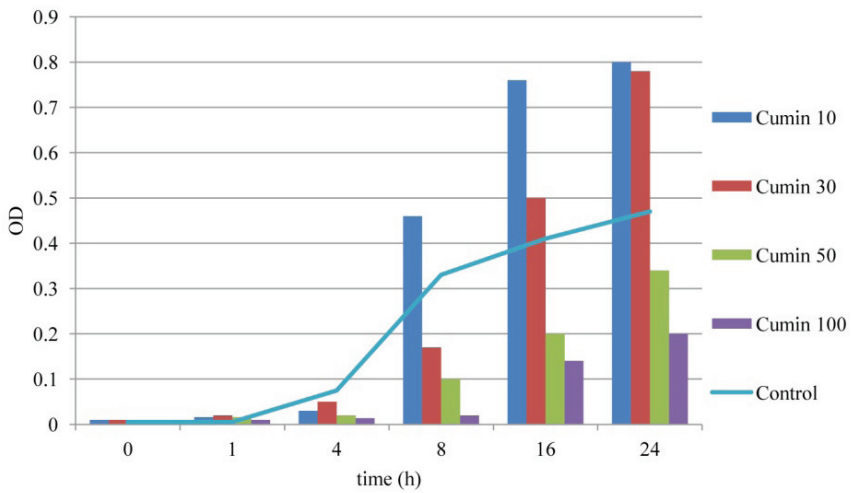

Figure 1. Effect of cumin essential oil on *E. coli* growth kinetics

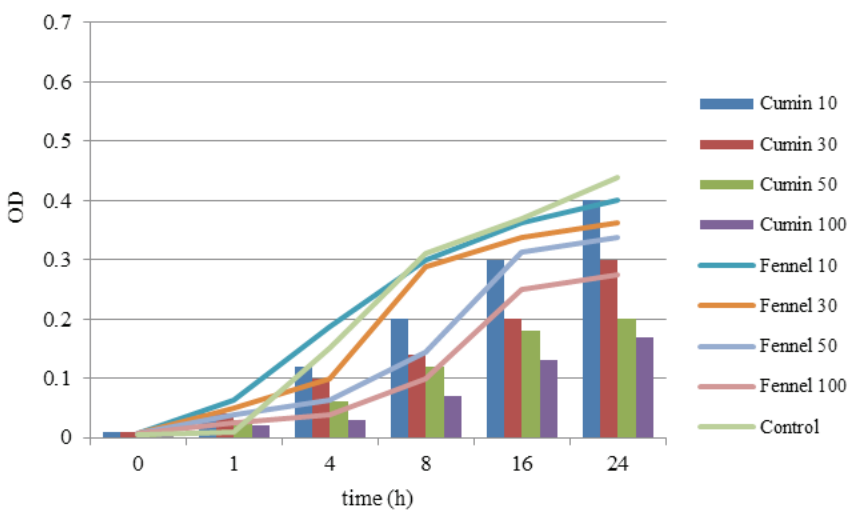

Figure 2. Effect of cumin and fennel essential oils on *C. albicans* growth kinetics

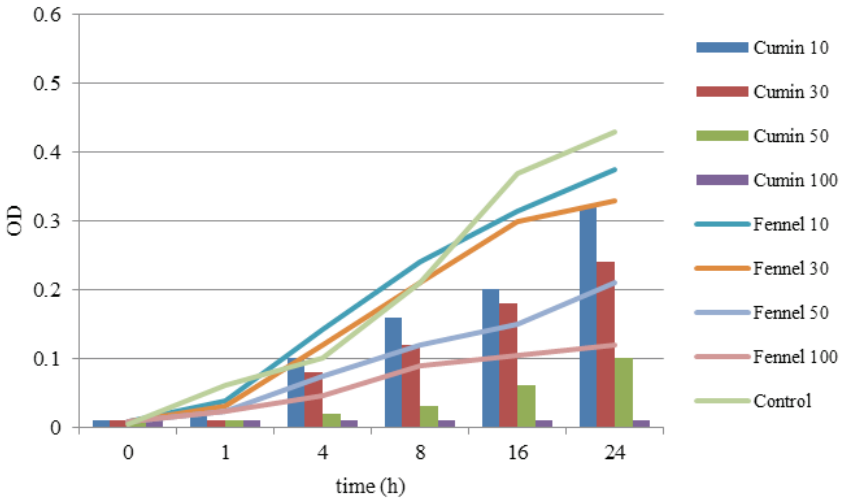

Figure 3. Effect of cumin and fennel essential oils on *S. aureus* growth kinetics

were about 100 µg/ml for Gram-positive bacteria *S. aureus* and *C. albicans*, and more than 100 µg/ml for Gram-negative bacteria *E. coli*. EO of cumin exhibited bacteriostatic effect against all tested microorganisms. Iacobellis et al. (2005) reported that cumin EO is more effective against *S. aureus* than against *E. coli* in the antibacterial screening with disk diffusion method, that was confirmed by our study as well. This was expected, because the Gram-negative bacteria has an outer membrane, surrounding its cell wall, that makes them more resistant to EO than Gram-positive bacteria. Burt (2004) reported that EOs with high antibacterial activity contain phenolic compounds as main constituents. EO of cumin contain such compounds, p-cymene, α-pinene, limonene, that explain its significant antimicrobial effect showed in this study. Cumin EO also showed an antifungal effect, that is very well documented in the previous studies (Iacobellis et al., 2005; Özcan and Erkmen, 2001; Singh et al., 2006a).

The results of antimicrobial analyses showed much stronger antimicrobial activity of cumin EO than fennel EO, that is also previously documented (Pai et al., 2010; Özcan and Erkmen, 2001). Also, EO of fennel showed antibacterial effect only against *S. aureus* and *C. albicans* among all tested microorganisms. It is probably due to the content of monoterpenes p-cymene, limonene and α-pinene in the cumin oil in much higher concentration than in the fennel EO.

Both essential oils were ineffective to prevent the growth of *E. facealis* at all tested concentrations (Table 2).

Figure 3 shows the significant inhibition of *S. aureus* growth with cumin EO at concentrations around 100 µL/ml, and with fennel EO at more than 100 µL/ml. These concentrations were considered for their MIC values. *C. albicans* showed less sensitivity on fennel EO, which in defining concentrations does not result in total inhibition of growth of this pathogenic fungus (Figure 2). Slightly stronger antifungal activity in applied concentrations is show by cumin EO, with MIC value around 100 µl/ml. *E. coli* showed higher resistance to applying concentrations of cumin EO (Figure 1) and the MIC value was also more than 100 µL/ml. These results were partially expected considering preliminary results of inhibition zones to used microorganisms (Table 2).

The results of antimicrobial activity of cumin and fennel essential oil in this study were different from the results of earlier reports (Lo Cantore et al., 2004; Ruberto et al., 2000; Diao et al., 2014; Eikani et al., 2007). This difference in MIC may be due to the difference in chemical composition of the essential oil, bacterial strains that were tested, methods of antibacterial analyses and the medium used.

CONCLUSION

The present study demonstrates that plants of these two Apiaceae species have noticeable antibacterial activity and potential applications as natural antibiotics. The antibacterial screening highlights that, among these two tested species, the EO of cumin had stronger antibacterial activity against tested microorganisms. Therefore, cumin essential oil could be very interesting in further investigations designing avaible *Drug delivery systems* with this essential oil encapsulated.

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