No. 4
673 - 676

Antimicrobial Activity and Chemical Composition of the Essential Oils of Portuguese *Foeniculum vulgare* Fruits

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Received: December 18th, 2014; Accepted: February 11th, 2015

The aim of this study was to investigate the chemical composition and antimicrobial activity of essential oils obtained by hydrodistillation from fruits of six fennel accessions collected from wild populations occurring in the centre and south of Portugal. Composition of essential oils was established by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The obtained yields of the essential oils were found to vary greatly in the range of 1.1 to 2.9% (v/w) and the chemical composition varied with the region of collection. A total of 16 compounds were identified. The main compounds were fenchone (16.9 – 34.7%), estragole (2.5 – 66.0%) and *trans*-anethole (7.9 – 77.7%). The percentages of these three main compounds were used to determine the relationship between the different oil samples and to group them into four different chemotypes: anethole/fenchone; anethole; estragole and anethole/estragole. Antifungal activity of essential oils was evaluated against six food spoilage fungi: *Aspergillus niger, A. japonicus, A. oryzae, Fusarium oxysporum*, *Rhizophus oryzae* and *R. stolonifer*. Antibacterial activity was assessed against three Gram-positive strains: *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228 and *S. aureus* ATCC 28213; and against six Gram-negative strains: *Escherichia coli* ATCC 25922; *Morganella morganii* LFG 08; *Proteus mirabilis* LFG 04*; Salmonella enteritidis* LFG 05; *S. entiritidis serovar typhimurium* LFG 06 and *Pseudomonas aeruginosa* ATCC 27853 by the disc diffusion agar method; the minimal inhibitory concentration (MIC) was determined using the broth macro-dilution method. The MIC values varied from 62.5 (*E. coli* ATCC 25922) to 2000 µg/mL (*P. aeruginosa* ATCC 27853).

Keywords: *Foeniculum vulgare*, Essential oils, Fruits, Chemical composition, Chemotypes, Antimicrobial activity.

Fennel (*Foeniculum vulgare* Mill.), family *Apiaceae*, is native to the Mediterranean region. It is an annual, biennial or perennial plant, depending on the variety [1]. The fruits and essential oils are used in the cosmetic, pharmaceutical and food industries, such as flavoring agents in liqueurs, bread, pickles, pastries and cheese [2].

Two subspecies of *F. vulgare* occur in Portugal: *F. vulgare* subsp. *capillaceum* and *F. vulgare* subsp. *piperitum* [3]. Previous studies on Portuguese fennel essential oils [3, 4, 5] have already reported the existence of chemotypes (anethole, anethole/fenchone, anethole/estragole) that deviate from the international accepted standards [6, 7, 8]. For instance, three chemotypes were described by Bernáth, namely anethole/estragole, anethole and estragole [8]. Chemical polymorphism is characteristic of this species and several factors such as climatic and environmental conditions, harvesting season and the stage of seed ripening can influence the content of essential oil and its composition in fennel [6, 7, 9, 10].

Since ancient times plants and essential oils were used for the treatment of infectious diseases even though no knowledge about microorganisms existed [11]. Nowadays, the increasing resistance of pathogens to antibiotics presents a major threat to public health because it reduces the effectiveness of treatment, which could lead to an increase in morbidity and mortality [12-14]. For instance, the emergence of resistance in the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* has already been documented [15]. Hence, there is an urgent need to find alternative antimicrobial agents to fight resistant pathogenic microorganisms. Although the most important sources of antibiotics are moulds, actinomycetes and bacteria, higher plants also contain many classes of secondary metabolites with antimicrobial properties, thus, efforts are being made to evaluate the antimicrobial activity of a wide variety of natural products, including plant metabolites, in order to isolate and characterize novel compounds which could inhibit bacteria and fungi, or even serve as models for new molecules [12]. Previous studies have already shown that fennel essential oils possess antimicrobial activity against several microorganisms [16-22].

As part of our continuous search for improving the knowledge about the germplasm of fennel in Portugal, six indigenous accessions were collected from wild populations growing in the centre (Guarda, Leiria and Santarém) [23] and south (Évora) of Portugal, and the seed progeny were evaluated for antimicrobial activity and variability in essential oils composition. The yields of the essential oils obtained from *F. vulgare* fruit samples varied greatly within a range of 1.1 to 2.9%, v/w (Table 1).

Table 1: Collection data and essential oil yields of analyzed fennel samples (*Foeniculum vulgare*).

Accessions	Location	Yield % (v/w)	Chemotype
09564	Guarda	2.5	Anethole/Fenchone
09576	Guarda	2.9	Anethole
09578	Leiria	1.6	Estragole
09583	Santarém	1.1	Anethole/Estragole
09586	Santarém	1.9	Anethole/Estragole
09873	Évora	1.1	Anethole