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Foeniculum vulgare Essential Oils: Chemical Composition, Antioxidant and Antimicrobial Activities

Maria Graça Miguel[a*], Cláudia Cruz[a], Leonor Faleiro[b], Mariana T. F. Simões[c], Ana Cristina Figueiredo[c], José G. Barroso[c] and Luis G. Pedro[c]

[a]*Universidade do Algarve, Faculdade de Engenharia e Recursos Naturais, Centro de Desenvolvimento de Ciências e Técnicas de Produção Vegetal, Campus de Gambelas 8005-139 Faro, Portugal*

[b]*Universidade do Algarve, Faculdade de Engenharia e Recursos Naturais, IBB, Centro de Biomedecina Molecular e Estrutural, Campus de Gambelas 8005-139 Faro, Portugal*

[c]*Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, IBB, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749-016 Lisbon, Portugal*

mgmiguel@ualg.pt

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The essential oils from *Foeniculum vulgare* commercial aerial parts and fruits were isolated by hydrodistillation, with different distillation times (30 min, 1 h, 2 h and 3 h), and analyzed by GC and GC-MS. The antioxidant ability was estimated using four distinct methods. Antibacterial activity was determined by the agar diffusion method. Remarkable differences, and worrying from the quality and safety point of view, were detected in the essential oils. *trans*-Anethole (31-36%), α-pinene (14-20%) and limonene (11-13%) were the main components of the essentials oil isolated from *F. vulgare* dried aerial parts, whereas methyl chavicol (= estragole) (79-88%) was dominant in the fruit oils. With the DPPH method the plant oils showed better antioxidant activity than the fruits oils. With the TBARS method and at higher concentrations, fennel essential oils showed a pro-oxidant activity. None of the oils showed a hydroxyl radical scavenging capacity >50%, but they showed an ability to inhibit 5-lipoxygenase. The essential oils showed a very low antimicrobial activity. In general, the essential oils isolated during 2 h were as effective, from the biological activity point of view, as those isolated during 3 h.

Keywords: *Foeniculum vulgare*, Essential oil, Phtytoceuticals, Biological activities.

Fennel (*Foeniculum vulgare* Mill.), (Umbelliferae / Apiaceae), is an annual, biennial or perennial herbaceous plant, depending on the variety, which grows in good soils from sunny mild climatic regions. Two subspecies are known, *F. vulgare* Mill. ssp. *capillaceum* (= *F. vulgare* Mill. ssp. *vulgare*) and *F. vulgare* Mill. ssp. *piperitum*. The subspecies *capillaceum* includes three varieties: *azoricum* Mill. Thell. (Florence), *dulce* Mill. Thell. (sweet) and *vulgare* Mill. Thell. (bitter).

F. vulgare is cultivated in every country surrounding the Mediterranean Sea because of its flavor [1-5]. Several fennel parts are edible (bulbs, leaves, stalks and fruits), and medicinally the plant is used as a secretomotor, secretolytic and antiseptic expectorant, spasmolytic, carminative, galactagogue for lactating women and as an eye lotion. Fruits (commonly known as seeds), and their essential oils, are used for savory formulations, for sauces and liqueur production, as well as an ingredient of cosmetics and pharmaceutical products. Fennel infusions are the classical tea for nursing babies to prevent flatulence and spasms [3,6,7]. Dried aerial parts and fruits of fennel are sold in herbal shops for household use, such as teas or to condiment food. It is thus of extreme importance that the safety, quality and efficacy of these herbal products is assessed, namely when some of its constituents are suspected to be harmful.

The essential oils of the fruits (seeds) are composed of a mixture of several monoterpenes and phenylpropanoids, with *trans*-anethole, methyl chavicol (= estragole), fenchone and limonene as main constituents [8]. The relative percentages of these components in the essential oils depend on the subspecies (*vulgare* and *piperitum*), varieties and chemotypes, different plant parts, ontogenesis, origin, environmental conditions, and type and conditions of extraction [1,2,4-6,8-18]. Previous studies have already shown that fennel oils

Table 1: Percentage composition of the essential oils isolated by hydrodistillation, for different periods, from *Foeniculum vulgare* aerial parts and fruits.

Components	RI	*Foeniculum vulgare*							
		Aerials parts				Fruits			
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h
α-Thujene	924	0.1	0.2	t	0.3	t	t	t	t
α-Pinene	930	**14.2**	**13.9**	**19.8**	**16.4**	0.4	0.8	1.2	1.4
Camphene	938	0.7	0.8	0.9	1.0	t	t	0.1	0.1
Sabinene	958	t	t	t	t	t	t	t	t
1-Octen-3-ol	961	t	t	t	t	t	t	t	t
β-Pinene	963	4.4	4.5	4.9	4.4	t	0.3	0.3	0.3
Dehydro 1,8-cineol	973	0.1	0.2	0.2	0.3	t	t	t	t
2-Pentyl furan	973	0.1	t	t	t	t	t	t	t
Myrcene	975	3.5	3.8	2.1	3.8	t	0.3	0.4	0.4
α-Phellandrene	995	5.1	5.1	5.7	2.5	t	0.2	0.1	t
δ-3-Carene	1000	t	t	t	t	t	t	t	t
Benzene acetaldehyde	1002	t	t	t	t	t	t	t	t
p-Cymene	1003	7.3	7.2	7.7	7.9	t	0.1	0.1	0.1
α-Phellandrene	1005	0.6	0.5	0.5	t	t	0.6	0.7	t
Limonene	1009	**10.7**	**11.9**	**12.5**	**13.1**	**6.9**	**10.0**	**12.3**	**12.2**
cis-β-Ocimene	1017	0.9	0.9	0.9	1.2	0.3	0.8	0.4	0.4
trans-β-Ocimene	1027	t	t	t	t	t	t	t	t
γ-Terpinene	1035	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.4
Fenchone	1050	5.6	5.6	5.5	5.3	3.5	4.0	4.1	3.9
Terpinolene	1064	0.1	0.1	0.1	0.1	t	t	0.1	t
n-Nonanal	1073	t	t	t	t	t	t	t	t
endo-Fenchol	1085	0.1	t	t	0.1	t	t	t	t
Oct-1-en-3-ol acetate	1086	0.1	t	t	1.1	t	t	t	t
Camphor	1095	0.1	0.1	0.1	t	t	t	0.1	t
trans-β-Limonene oxide	1112	0.1	0.1	0.1	0.2	t	t	t	0.1
cis-Verbenol	1110	0.1	t	t	t	t	t	t	t
trans-Verbenol	1114	0.3	0.3	0.2	0.1	t	t	t	0.1
Pinocarvone	1121	t	t	t	t	t	t	t	t
p-Mentha-1,5-dien-8-ol*	1137	0.3	0.1	0.3	0.3	t	t	t	t
Terpinen-4-ol	1148	0.4	0.4	0.4	0.4	t	t	t	0.1
Octanoic acid	1156	0.3	0.1	0.2	0.1	t	t	t	t
α-Terpineol	1159	0.2	t	t	t	t	t	t	t
Methyl chavicol (= estragole)	1163	5.2	4.7	3.8	3.9	**88.0**	**82.0**	**79.3**	**79.3**
Myrtenol	1168	t	t	t	t	t	t	t	t
trans-Carveol	1189	0.3	0.3	0.3	0.3	t	t	t	t
p-Anisaldehyde*	1200	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Carvone	1206	0.1	0.2	0.2	0.2	0.1	0.1	t	0.1
2-Methyl butyric acid hexyl ester	1220	t	t	t	t	t	t	t	t
cis-Anethole	1220	t	t	t	t	t	t	t	t
Hexyl isovalerate	1225	0.1	t	0.1	t	t	t	t	t
Geraniol	1236	t	t	t	0.1	t	t	t	t
***trans*-Anethole**	1254	**35.6**	**36.0**	**30.7**	**32.3**	0.5	0.4	0.4	0.5
Carvacrol	1286	0.9	0.8	1.2	2.0	t	t	t	t
ar-Curcumene	1475	t	0.1	0.1	0.1	t	t	t	t
β-Sesquiphellandrene	1508	t	t	t	t	t	t	t	t
Geraniol 2-methyl butyrate	1586	t	t	t	t	t	t	t	t
Geraniol isovalerate	1590	t	t	t	t	t	t	t	t
Tetradecanoic acid	1723	0.2	0.2	0.2	0.5	t	t	t	t
Hexadecanoic acid	1908	t	t	t	0.1	t	t	t	t
Phytol acetate	2047	t	t	t	t	t	t	t	t
% of identified components		98.0	98.4	99.0	98.5	100.0	100.0	99.9	99.5
Grouped components									
Monoterpene hydrocarbons		47.7	49.0	55.2	50.9	7.8	13.4	15.9	15.3
Oxygen containing monoterpenes		8.6	8.1	8.5	9.3	3.6	4.1	4.2	4.3
Sesquiterpene hydrocarbons		t	0.1	0.1	0.1	t	t	t	t
Phenylpropanoids		40.9	40.9	34.7	36.4	88.6	82.5	79.8	79.9
Others		0.8	0.3	0.5	1.8	t	t	t	t
Oil Yield (v/w)		0.23	0.20	0.29	0.29	0.31	0.31	0.63	0.87

RI, Retention Index relative to C_9-C_{21} *n*-alkanes on the DB1 column; t, trace (<0.05%); *identification based on mass spectra only.

possess antioxidant ability, as well as antimicrobial activity against several microorganisms of animal and human origin, and plant pathogens, including fungi [11,19-27]. Studies concerning the effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial effects of essential oils

are, nevertheless, limited. Thus, in addition to evaluating the quality of the essential oils from commercially available fennel preparations (phytoceuticals), the aim of this work was also to assess the effect of four different hydrodistillation times (30 min, 1 h, 2 h and 3 h) on oil yields, composition, and antimicrobial and antioxidant activities.

Composition of the essential oils

The relative amounts of the volatile components identified in the essential oils isolated from fennel samples, obtained after different distillation times, are listed in Table 1, in order of their elution from a DB-1 column. The highest oil content was obtained from fruits after 3 h of hydrodistillation. In this case, the oil content was three times higher (0.9%, v/dry wt.) than that obtained from fruits extracted for 30 min and 1 h (0.3%). This difference was not observed in the essential oils isolated from the fennel aerial parts, since there was only a one fold increase in the oil yield between 30 min and 3 h of hydrodistillation.

Different oil contents were found by Ravid *et al*. [9] after 2 h of distillation of Israeli sweet fennel leaves, stems, and unripe and ripe umbels, in two different developmental phases. The maximum yield was obtained from ripe umbels in the early fruiting stage (3%, v/w) and the minimum from leaves in the late fruiting stage (0.1%). According to Miraldi [14], the age of the fruit samples, as well as incorrect storage conditions, such as open containers, exposure to light and excessive temperature, among other factors, may negatively affect fennel oil yield.

Although the monoterpene fraction was dominant (56-64%) in the essential oil isolated from fennel aerial parts, the main component was the phenyl-propanoid *trans*-anethole (31-36%), followed by α-pinene (14-20%) and limonene (11-13%) (Table 1).

Piccaglia and Marotti [16] indicated the presence of five different chemical groups in the oils isolated from fresh aerial parts of wild fennel collected in thirteen Italian localities: 1) *trans*-anethole, methyl chavicol, α-phellandrene; 2) *trans*-anethole, α-pinene, limonene; 3) methyl chavicol, α-phellandrene; 4) methyl chavicol, α-pinene; and 5) α-phellandrene. Based on the relative amounts of *trans*-anethole, α-pinene and limonene found in the essential oils isolated from the aerial parts in the present study, we can include these in group 2, as defined by Piccaglia and Marotti [16].

Of high relevance, but also of major concern, was the chemical composition of fennel fruits. The phenylpropanoid fraction (80-89%) and methyl chavicol (= estragole) (79-88%), dominated the fruit oil. The relative amounts of *trans*-anethole in these oils were much lower than those that characterize bitter fennel oils [28].

Some previous studies on fennel fruit essential oils have also mentioned methyl chavicol chemotypes in variable amounts, where methyl chavicol alone dominates the oil, or is present together with either *trans*-anethole or fenchone (Table 2). The methyl chavicol contents of the essential oils from fruits analyzed in the current study (79-88%) were higher than those reported in the literature for methyl chavicol-rich fruits oils (Table 2).

Considering that fennel fruits are of common use, both as seasoning, in children's nourishment and to relieve several ailments, the fact that preparations with this high methyl chavicol content are commercially available, raises the question of product safety and of the need to create standardized products.

The influence of increasing the distillation time on the chemical composition of the essential oils was more evident in the variation of the relative amounts of limonene and methyl chavicol (Table 1). Whereas limonene showed a rising tendency, both in the aerial parts (11% to 13%) and fruit oils (7% to 12%), methyl chavicol showed the opposite behavior (5% to 4% and 88% to 79% in the aerial parts and fruit oils, respectively). The decrease in the relative amount of methyl chavicol with increasing distillation time may be

Table 2: Methyl chavicol (= estragole) rich *Foeniculum vulgare* fruits essential oils and volatiles*,**, reported in the literature.

Species	Country	Isolation procedure	Chemotype	Methyl chavicol average amount	Reference
Foeniculum vulgare	Egypt	Steam distillation	Methyl chavicol / *trans*-anethole	47%	[29]
Foeniculum vulgare subsp. *piperitum*	Portugal	Hydrodistillation	*trans*-Anethole / methyl chavicol	36%	[10]
Foeniculum vulgare subsp. *capillaceum*	Italy and France	Hydrodistillation	Methyl chavicol and *trans*-anethole / methyl chavicol	37-43%	[12]
Foeniculum vulgare	Spain	Pentane extraction	Methyl chavicol / fenchone	65%	[30]
Foeniculum vulgare	Germany	Hexane extraction	Methyl chavicol	33-73%	[31]
Foeniculum vulgare var. *vulgare*	Israel	Hexane extraction	Methyl chavicol	63%	[13]
Foeniculum vulgare	Spain	Hydrodistillation	Methyl chavicol / fenchone	55%	[15]
Foeniculum vulgare	Italy	Steam distillation	Methyl chavicol	35-50%	[16]
Foeniculum vulgare var. *vulgare*	Israel	Hexane extraction	Methyl chavicol	42-61%	[8]
Foeniculum vulgare subsp. *piperitum*	Turkey	Hydrodistillation	Methyl chavicol / fenchone	53-62%	[18]

* fruits were extracted with pentane, ** fruits were extracted with hexane

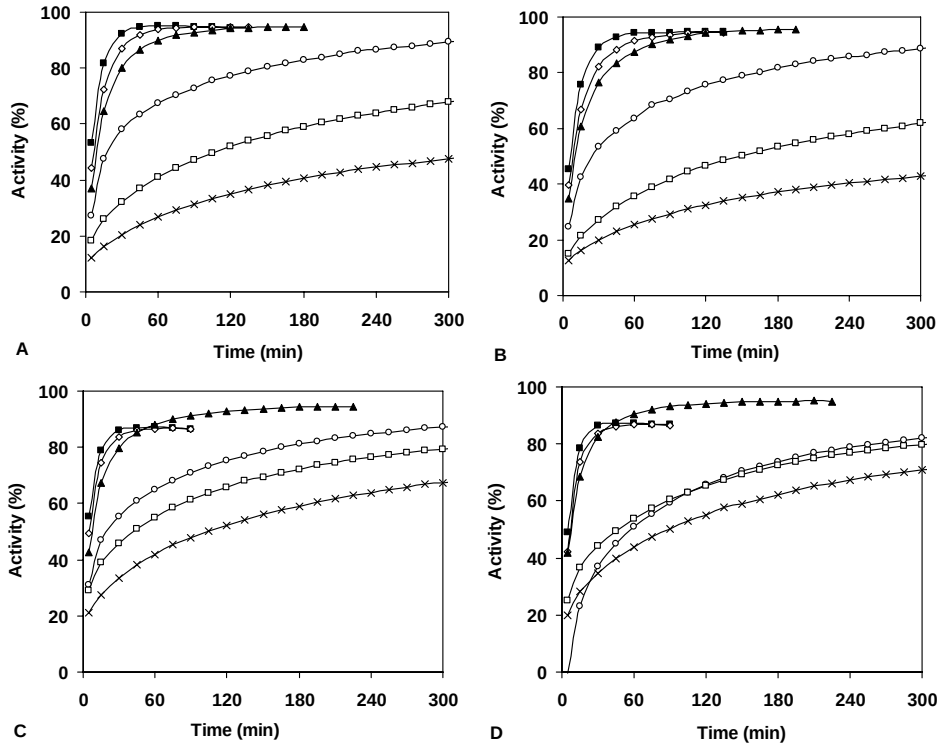

Figure 1A-D: Time-course evaluation of the free radical scavenging activity, from different concentrations, of the essential oils (■ 24 g/L, ◇ 18 g/L, ▲ 12 g/L, ○ 6 g/L, □ 3 g/L, ✕ 1 g/L), isolated from *Foeniculum vulgare* dried aerial parts, with different hydrodistillation times: 30 min (A), 1 h (B), 2 h (C) and 3 h (D).

partially attributable to its higher water solubility than that of limonene.

The results for the chemical composition of the essential oils of fennel aerial parts and fruits support the view of Miraldi [14] that knowledge on fennel essential oils is still not enough to distinguish accurately all the existing varieties.

Antioxidant activity

There is not a single method to evaluate a sample as a possible antioxidant, since the different methods used to determine this activity often give different results, depending on the reaction mechanism involved in the method. Also very important is the selection of an appropriate reference for each method in order to compare the potential antioxidant activities of the samples [32]. Taking into account these premises, in the present work, different methods of antioxidant activity evaluation were performed, always against an appropriate positive control: TBARS method that measures the formation of secondary (malonaldehyde) components of the oxidative process of lipids; DPPH radical scavenging capacity assay that measures the capacity of the sample to reduce the stable organic nitrogen radical; hydroxyl radical scavenging capacity and inhibition of lipoxygenase, responsible for the oxidation of arachidonic acid.

Free radical scavenging activity (DPPH): The time-course evaluation of the free radical scavenging capacity of the different concentrations of the essential oils, isolated using different hydrodistillation periods, from the dried aerial parts of fennel (Figure 1 A-D), showed, in all cases, that the higher concentrations of the essential oils (12-24 g/L) attained the steady-state faster and had better DPPH free radical scavenging ability. However, steady-state, as well as 85-95% of activity, was only attained at the end of 30 min. In general, at the highest concentrations assayed (12-24 g/L), the essential oils from the different distillation periods showed similar DPPH free radical scavenging capacity. Whereas at the highest concentrations assayed (12-24 g/L), the antioxidant capacity was >85%, for the lowest concentrations (2-6 g/L), only the essential oils isolated during 2 h and 3 h of hydrodistillation showed an antioxidant capacity >60%, and only after 6 h of reaction.

The fruits oils (Figure 2 A-D) showed lower antioxidant ability than those of the aerial parts. Antioxidant capacities of 85-95% were attained only after 2 h of reaction, with the highest oil concentrations (24 g/L) and with oils obtained after 2 h and 3 h of hydrodistillation.

The antioxidant ability of fennel essential oil samples was lower than that of the positive control, α-tocopherol.

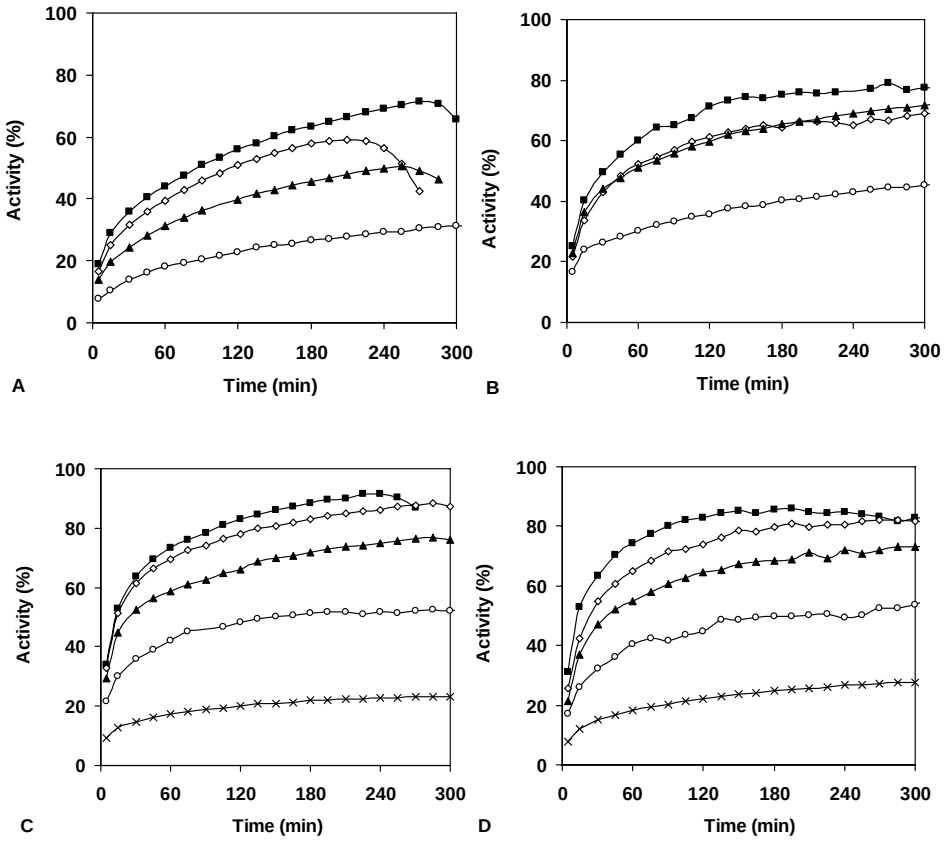

Figure 2A-D: Time-course evaluation of the free radical scavenging activity of different concentrations of the essential oils (■ 24 g/L, ◇ 18 g/L, ▲ 12 g/L, ○ 6 g/L, □ 3 g/L, ✕ 1 g/L), isolated from *Foeniculum vulgare* fruits, with different hydrodistillation times: 30 min (A), 1 h (B), 2 h (C) and 3 h (D).

At lower concentrations (5-100 mg/L), α-tocopherol reached the steady-state in 15-30 min with an antioxidant capacity of 35-96%.

Sánchez-Moreno *et al*. [33] classified the kinetic behaviour of an antioxidant compound as rapid (<5 min), intermediate (5-30 min) and slow (>30 min), based on the time required to reach a steady state at the concentration corresponding to a 50% decrease of the initial $DPPH^{\bullet}$ concentration (TEC_{50}). For ascorbic acid and rutin, the values of TEC_{50} were 1 min and 103 min, respectively. Some components also react reversibly with DPPH, such as eugenol and other phenols bearing a similar structure type (*o*-methoxyphenol), giving false low readings [32]. Fennel essential oils evaluated in the present work should thus be classified as slow in terms of kinetic behavior.

Politeo *et al*. [26] reported a weak capacity of fennel essential oils for scavenging free radicals. Using the DPPH method and reading the absorbance decay after 1 h, these authors referred inhibitory percentages of 2-9% for concentrations ranging from 5 to 50 g/L. In the present work, after 1 h and at the highest concentration tested (24 g/L), the antioxidant activity varied from 87-94%, depending on the distillation time. These values were more than 10 fold higher than those reported by Politeo *et al*. [26] for *trans*-anethole dominant fennel oils (78%), which was about double the amount found in the aerial parts of the fennel oils analyzed in the present study (31-36%). Purtas-Mejía *et al.* [20] also referred to the weak antioxidant activity of *trans*-anethole dominant fennel essential oils (80%), which in that study was the worst oil of the seven oils examined.

Thiobarbituric acid reactive substances (TBARS): At the lowest fennel oil concentrations tested (100 and 250 mg/L), the fruits oils showed always lower activity than the oils obtained from the aerial parts (Table 3). Nevertheless, at the higher concentration, the fruit oil's activity was either similar to or higher than that of the oil from the aerial parts. The differences in antioxidant activity between the different concentrations assayed of the oils from the aerial parts were not as pronounced as those observed for the fruits oils.

As the concentrations of the oils of the aerial parts >750 mg/L there was a decrease in the antioxidant activity. This behavior was also observed for the seed oils, but generally at oil concentrations >1000 mg/L. These results may indicate that high concentrations of fennel essential oils possess a pro-oxidant activity, independent of their richness in *trans*-anethole or methyl chavicol.

Table 3: Antioxidant index (%), determined by the TBARS method, of different concentrations of the essential oils isolated from *Foeniculum vulgare*, with different hydrodistillation times, and of the positive control (α-tocopherol).

Hydrodistillation time	*Foeniculum vulgare*				α-Tocopherol	
	Aerial parts		Fruits			
	Concentration (mg/L)	Activity (% (mean ± SD n=3)	Concentration (mg/L)	Activity (%) (mean ± SD n=3)	Concentration (mg/L)	Activity (%) (mean ± SD n=3)
30 min	2000	57.3±2.5	2000	74.8±3.5	1000	86.0±1.0
	1500	56.4±2.8	1500	76.1±4.2	500	83.7±2.4
	1000	71.3±3.5	1000	66.1±3.8	250	83.0±4.6
	750	69.9±0.5	750	66.5±1.1	100	74.4±6.0
	500	70.8±1.2	500	52.2±0.8	50	72.4±4.6
	250	64.9±0.7	250	37.7±3.9	10	35.3±6.0
	100	54.4±1.7	100	9.7±2.0	5	18.4±4.8
1 h	2000	56.4±1.1	2000	56.1±4.1		
	1500	61.6±0.3	1500	57.8±1.1		
	1000	70.3±0.0	1000	79.5±0.5		
	750	73.2±2.0	750	78.4±2.9		
	500	64.1± 2.8	500	70.5±4.0		
	250	68.3±3.2	250	47.2±0.8		
	100	56.0±2.5	100	21.9±1.4		
2 h	2000	54.6±1.1	2000	57.1±2.0		
	1500	54.1±2.1	1500	58.5±4.9		
	1000	57.1±1.4	1000	70.1±2.6		
	750	67.3±1.4	750	72.0±1.2		
	500	63.0±5.6	500	61.5±0.4		
	250	63.0±3.2	250	38.2±2.2		
	100	54.1±6.6	100	14.6±3.2		
3 h	2000	54.5±1.7	2000	49.7±4.5		
	1500	53.6±2.9	1500	56.9±3.8		
	1000	58.8±2.9	1000	65.9±3.2		
	750	61.4±0.3	750	58.6±1.8		
	500	65.6±1.0	500	58.0±3.6		
	250	64.8±1.8	250	38.4±5.6		
	100	55.1±3.1	100	19.1±3.6		

The antioxidant activity of the positive control (α-tocopherol) was dose-dependent from 50 to 100 mg/L, after which stabilization was achieved. α-Tocopherol showed higher antioxidant capacity than the fennel oil samples, since either 500 mg/L or 750 mg/L of fennel oil samples, obtained after 30 min or 1 h of hydrodistillation, were needed to achieve similar activity to that of α-tocopherol at 50 mg/L.

The antioxidant activities determined in the present study were similar to those reported by Ruberto *et al.* [19] for two methyl chavicol-rich fennel oils (53 and 58%). Nevertheless, at 100 and 250 mg/L the antioxidant activities determined in the present study were lower than those reported by those authors [19]. This may eventually be due to the higher levels of this compound in the current study (79-88%).

Testing the antioxidant activities of pure standards, with the TBARS method, Ruberto and Baratta [34] showed that the activities of methyl chavicol ranged from 5%, at 100 mg/L, to 32%, at 1000 mg/L, whereas with *trans*-anethole, the values found were 30 and 42%, respectively. Therefore, methyl chavicol seems to possess weaker activity than *trans*-anethole. With the latter compound, different concentrations did not greatly influence the antioxidant activity.

Hydroxyl radical scavenging activity: In contrast to what was found for the lipid peroxidation assay, in which the major differences between the lowest and the highest oil concentrations were more evident in fruit oils, in the hydroxyl radical scavenging activity assay (Table 4) such pattern was detected in the oils from the aerial parts of fennel, but never as pronounced as in TBARS method.

Within the concentrations assayed, neither the oils of the aerial parts nor the fruit showed a hydroxyl radical scavenging capacity >50%. The lowest concentration of mannitol assayed (1.8 g/L) showed similar hydroxyl radical scavenging capacity to that of fennel oils in similar concentrations. Higher concentrations of mannitol showed higher activities (74%).

Hinneburg *et al*. [35] reported the capacity of fennel extracts for scavenging hydroxyl radicals, but their results cannot be correlated with the present ones, since the extraction procedure, as well as the units used, were different.

Table 4: Hydroxyl radical scavenging activity, determined by the deoxyribose method, of different concentrations of the essential oils isolated from *Foeniculum vulgare* with different hydrodistillation times, and of the positive control (mannitol).

Hydrodistillation time	*Foeniculum vulgare*				Mannitol	
	Aerial parts		Fruits			
	Concentration (mg/L)	Activity (%) (mean ± SD n=3)	Concentration (mg/L)	Activity (%) (mean ± SD n=3)	Concentration (mg/L / mM)	Activity (%) (mean ± SD n=3)
	2000	42.0±0.4	2000	44.9±1.7	91100 / 0.5	74.0±2.8
	1500	41.9±0.9	1500	35.9±0.3	18220 / 0.1	66.5±1.6
	1000	42.0±1.8	1000	47.5±1.3	1822 / 0.01	48.8±0.6
30 min	750	40.3±0.2	750	47.8±0.6		
	500	40.0±0.2	500	44.5±0.6		
	250	38.7±2.3	250	43.7±0.6		
	100	35.2±1.2	100	39.5±2.9		
	2000	38.2±0.5	2000	44.6±3.6		
	1500	40.1±1.7	1500	41.9±1.9		
	1000	39.6±1.4	1000	41.7±1.9		
1 h	750	36.6±1.0	750	42.5±1.8		
	500	37.7±1.7	500	42.3±1.4		
	250	32.0±1.5	250	41.2±2.0		
	100	23.9±3.4	100	39.2±2.5		
	2000	43.3±1.3	2000	43.7±2.85		
	1500	41.2±2.3	1500	44.4±1.9		
	1000	44.4±1.4	1000	44.8±2.0		
2 h	750	39.1±3.2	750	40.6±1.5		
	500	41.9±2.1	500	41.1±0.7		
	250	38.5±0.8	250	41.9±1.0		
	100	29.6±1.1	100	33.7±6.0		
	2000	42.1±2.2	2000	43.2±1.8		
	1500	41.6±4.1	1500	44.5±1.6		
	1000	43.1±1.86	1000	42.1±0.6		
3 h	750	44.2±0.9	750	47.1±1.0		
	500	42.7±0.8	500	46.0±1.7		
	250	36.2±3.4	250	42.2±0.7		
	100	30.1±1.9	100	39.9 ±1.4		

Table 5: Percentage inhibition of 5-lipoxygenase with different concentrations of the essential oils isolated from *Foeniculum vulgare*, with different hydrodistillation times, and of the positive control, nordihydroguaiaretic acid (NDGA).

Hydrodistillation time	*Foeniculum vulgare*				Nordihydroguaiaretic acid (NDGA)	
	Aerial parts		Fruits			
	Concentration (mg/L)	Activity (%) (mean ± SD n=3)	Concentration (mg/L)	Activity (%) (mean ± SD n=3)	Concentration (mg/L / mM)	Activity (%) (mean ± SD n=3)
	250	89.4±1.1	250	95.6±2.1	151.2 / 0.5	98.0±0.7
30 min	100	48.6±0.6	100	31.0±1.6	30.2 / 0.1	46.8±2.4
	50	29.3±2.6	50	9.9±1.8	15.1 / 0.05	8.3±2.0
	250	91.6±2.2	250	98.3±0.4		
1 h	100	20.7±1.3	100	54.3±2.1		
	50	3.2±1.8	50	12.1±1.3		
	250	100.0±0.0	250	97.5±0.8		
2 h	100	54.4±2.6	100	49.1±2.1		
	50	10.7±2.1	50	23.9±0.7		
	250	92.2±1.1	250	91.9±2.8		
3 h	100	20.8±2.0	100	28.5±1.0		
	50	5.1±1.7	50	12.9±1.0		

5-Lipoxygenase assay: Although the essential oils isolated from fennel aerial parts and fruits showed ability to inhibit 5-lipoxygenase, higher concentrations than those of the positive control were needed to obtain the same activity (Table 5). As an example, 100 mg/L of fennel essential oils showed almost the same activity against 5-lipoxygenase as 30 mg/L NDGA. Stronger activities were obtained with the highest concentrations of the essential oils assayed (250 mg/L), independent of the type of essential oil and time of hydrodistillation.

It is noteworthy that the relative anti-inflammatory activity of essential oils of fennel seemed to be independent of the chemical composition of the the plant part used or hydrodistillation time. Likewise, Lourens *et al*. [36] reported remarkably similar activities of essential oils within the same plant genus, independent of their chemical composition, showing that combinations of different compounds may result in similar biological activities.

Antimicrobial activity

No significant antimicrobial activity was recorded against any of the tested microorganisms, except for *Salmonella* spp. Even for this Gram-negative bacterium the inhibition zone achieved was 8.33±0.58 mm for both the aerial parts and fruit essential oils. In the study of Sing *et al*. [21] the activity of the essential oil of *F. vulgare* also varied greatly between Gram-negative and Gram-positive bacteria. This low antimicrobial activity seems to be related to the strain susceptibility and the essential oil composition, which is poor on components that are usually related with higher antimicrobial activity, such as carvacrol and thymol [37].

In conclusion, the study herewith presented characterized the chemical composition, the antioxidant and antimicrobial activities of *F. vulgare* essential oils, isolated during different extraction periods, from commercial available products (phytoceuticals). In general, the essential oils isolated during 2 h were as effective, from the biological activity point of view, as those isolated during 3 h. In addition, the occurrence of high estragole contents raises the important question of quality control of herbal products and of the need to develop standardized products safe for human consumption.

Experimental

Plant material: *Foeniculum vulgare* products (dried aerial parts and fruits) were purchased from a local herbal shop.

Essential oil isolation: The essential oils were isolated from the dry plant material by hydrodistillation for 30 min, 1 h, 2 h and 3 h using a Clevenger-type apparatus, according to the European Pharmacopoeia method [38]. The essential oils were stored at -20ºC in the dark until analysis.

Gas chromatography (GC): Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) equipped with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m x 0.25 mm i. d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m x 0.25 mm i. d., film thickness 0.15 μm; J & W Scientific Inc.). Oven temperature was programmed from 45-175°C, at 3°C/min, subsequently at 15°C/min up to 300°C, and then held isothermal for 10 min; injector and detector temperatures, 280°C and 300°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.1 μL of a *n*-pentane-oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

Gas chromatography-mass spectrometry (GC-MS): The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph, equipped with a DB-1 fused-silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific, Inc.), and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, CT, USA). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40-300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{21} *n*-alkane indices and GC-MS spectra from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesised components and commercially available standards.

Antioxidant activity: From each sample, different concentrations of essential oils were prepared in methanol. The antioxidant activity of each sample was evaluated using 4 different methods: free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [39], thiobarbituric acid reactive substances assay (TBARS) [40,41], deoxyribose assay for scavenging of hydroxyl radicals (nonsite-specific hydroxyl radical-mediated deoxyribose degradation) [42], and by the 5-lipoxygenase assay [43]. All determinations were performed in triplicate. The results were stated in mean ± standard deviation.

Antimicrobial evaluation: In this study were used 4 different bacteria and 2 yeasts (*Candida albicans* ATCC 90028 and *Saccharomyces cerevisae*). The bacterial group included three Gram-positive bacteria, namely *Staphylococcus aureus* CFSA2, an environmental isolate, *Listeria monocytogenes* C882 (a food isolate from INETI-DTIA, Lisbon, Portugal), *Bacillus cereus* C1060 (a food isolate from INETI-DTIA, Lisbon, Portugal) and one Gram-negative species, *Salmonella* sp. (a gift from Regional Health Administration Service, Faro, Portugal). The antimicrobial activity was determined by the agar diffusion method, as previously described [44].

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