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Comparative Essential Oil Composition and Antifungal Effect of Bitter Fennel (*Foeniculum vulgare* ssp. *piperitum*) Fruit Oils Obtained During Different Vegetation

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ABSTRACT The chemical composition of the flower and unripe and ripe fruits from fennel (bitter) (*Foeniculum vulgare* ssp. *piperitum*) has been examined by gas chromatography and gas chromatography-mass spectrometry. The main identified components of the flower and unripe and ripe fruit oils were estragole (53.08%, 56.11%, and 61.08%), fenchone (13.53%, 19.18%, and 23.46%), and α-phellandrene (5.77%, 3.30%, and 0.72%), respectively. Minor qualitative and major quantitative variations for some compounds of essential oils were determined with respect to the different parts of *F. vulgare*. The oils exerted varying levels of antifungal effects on the experimental mycelial growth of *Alternaria alternata*, *Fusarium oxysporum*, and *Rhizoctonia solani*. The 40 ppm concentrations of fennel oils showed inhibitory effect against mycelial growth of *A. alternaria*, whereas 10 ppm levels were ineffective. The analyses show that fennel oils exhibited different degrees of fungistatic activity depending on the doses.

KEY WORDS: • ***antifungal effect*** • ***essential oil*** • ***estragol*** • ***fenchon*** • ***fennel (bitter)*** • **Foeniculum vulgare** • ***molds***

INTRODUCTION

BITTER FENNEL (*Foeniculum vulgare* ssp. *piperitum*), belonging to the Umbelliferae family, is a perennial or annual herbaceous and a typical aromatic plant that grows in several regions all over the world. It is growing to a height ranging from 70 to 200 cm.[1,2] It grows wild in most regions, especially the west and south regions of Turkey. Fresh or dried herb and fruits of bitter fennel (called "malotra" in Turkish) are used as a flavoring agent for some foods such as salad, cacık, and soup.

There are usually considerably variations in the major components within this species. Each variety because of morphological characteristics is known to possess a specific essential oil composition, with fenchone and *trans*-anethole being the most important components. The amounts of these components may vary in the oils of different origin. Specific estragole chemotypes are also known.[3,4]

Spices, herbs, and their derivatives such as essential oil and oleoresin are used in foods for their flavors and aroma. Recently, there has been considerable emphasis on studies involving essential oils and extracts of spices and their constituents for inhibiting the growth of microbes. It has also been known for some time that certain crude drugs and spices contain substances with antifungal activity in their derivatives.[5–7] Many of the spices and herbs were valued for their preservative and medical powers besides their flavor and odor qualities.[8–14] Although most of the reports on natural products in agricultural areas are about insects, there are important reports revealing that plant extracts and essential oils exhibit antimicrobial activity against food and cereal store fungi, leaf pathogens, and soilborne fungi.[15–17] Limited studies have been carried out on the composition and antifungal effect of essential oil of *Foeniculum vulgare* subsp. *piperitum* fruit of different maturation periods. This paper reports the results of composition and antifungal effect of oils obtained from flowers, unripe fruit, and ripe fruit of bitter fennel by hydrodistillation.

MATERIALS AND METHODS

Plant material

The aerial parts (flower and unripe and ripe fruits) of fennel (bitter) (*F. vulgare* ssp. *piperitum*) were collected in the Mersin (Büyükeceli-Gülnar) province in Turkey in July, August, and September 2003, and identified by the Laboratory of Systematic Botany, University of Selçuk, Konya, Turkey. A specimen has been deposited in the Food Engineer Museum of the University of Selçuk.

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Microorganisms

These fungi cause different infections in many plant cultivars and stored products that result in great economic loss.[18] The identified and pathogenically tested fungi used for this assay were obtained from the collections of the Plant Protection Department, Faculty of Agriculture, University of Selçuk.

Media

In order to obtain microorganism growth, the standard agar medium PDA was used [200 g of potato juice, 20 g of D(+)-glucose, 15 g of agar-agar, and 100 mL of distilled water]. Czapex Dox agar was used for the determination of fungal toxic effect (30 g of sucrose, 3 g of sodium nitrate, 0.5 g of magnesium sulfate, 1 g of potassium hydrogen phosphate, 13 g of agar, and 1,000 mL of distilled water).

Isolation of essential oil

Air-dried material (each about 100 g) were separately ground into small pieces and subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus; the oils obtained were dried over anhydrous sodium sulfate. Essential oil yield of the air-dried aerial parts of flower, unripe fruit, and ripe fruit *F. vulgare* subsp. *piperitum* as obtained by hydrodistillation was 2.08%, 6.01%, and 4.41%, respectively.

Identification of components

For identification of components, an analytical HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) was used with a DELSI 121 C apparatus fitted with a flame ionization detector and a CP WAX 51 fused silica column (25 m $\times$ 0.3 mm; 0.25 μm film thickness). The temperature was set at 50°C for 5 minutes and programmed to reach 220°C at a rate of 3°C/minute. A CP WAX 51 fused silica WCOT column (60 m $\times$ 0.3 mm) for gas chromatography was used with helium as carrier gas. For gas chromatography/mass spectrometry (GC/MS) a CP WAX 52 fused silica CB column (50 m $\times$ 0.25 mm) was used with helium as the carrier gas (flow rate 1 mL/minute) and coupled to an HP mass spectrometer with an ionization energy of 70 eV. Temperature programming was from 50° to 240°C at a rate of 3°C/minute. The samples were injected at an injector temperature of 240°C. The components were identified by comparing linear Kovats indices, their retention times, and mass spectra with those obtained from the authentic samples and/or the mass spectrometry library.

The percentage composition of the essential oils was computed from 6C peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and mass spectra with corresponding data in the literature.[19]

Assessment of inhibition of fungal growth

The effects of essential oils of different parts of fennel (bitter) were determined against *Alternaria alternata*, *Fusarium oxysporum*, and *Rhizoctonia solani* growth using Czapek-Dox agar medium only. Discs of the test fungi (5 mm i.d.), cut from the periphery of 7-day-old cultures, were inoculated separately onto each assay plate and incubated at 25°C for 7 days. Doses of 10 and 40 ppm of each essential oil were added on the sterile disc papers (10 mm i.d.), which were put into Petri plates. The colony diameter was then measured, and the percentage of mycelial inhibition was calculated following the procedure of Deans and Svoboda[7]:

$$I = [(C - T)/C] \times 100$$

where I is inhibition (%), C is the colony diameter of mycelium from a control Petri plate (in mm), and T is the colony diameter of mycelium from a test Petri plate (in mm). Three replicates of each treatment were carried out, and averages were calculated. Control sets were run simultaneously, using the medium without any essential oils.

RESULTS AND DISCUSSION

Chemical composition of oils

The components identified in the essential oils are listed in Table 1 in order of their experimental retention times and retention indices.

The essential oils exhibited light yellow color and typical fennel odor. The yields of the essential oil of dried flowers and unripe and ripe fruits from *F. vulgare* subsp. *piperitum* were 2.8%, 6.01%, and 4.41% (vol/wt), respectively. The essential oil yields of the three parts were found as different amounts. In this study, a total of 33, 24, and 20 compounds accounted for about 97.33%, 98.4%, and 99.15% of the essential oils of *F. vulgare*, respectively.

The major components of the different parts of *F. vulgare* were estragole, fenchone, α-phellandrene, and γ-terpinene. The main constituents of flowers oil were estragole (53.08%), α-phellandrene (5.77%), fenchone (13.53%), limonene + β-phellandrene (10.94%), exofenchyle acetate (3.27%), *p*-cymene (3.26%), and γ-terpinene (1.88%), while estragole (56.11%), fenchone (19.18%), limonene + β-phellandrene + 1,8-cineole (12.99%), α-phellandrene (3.30%), α-pinene (1.52%), and myrcene (1.19%) were established as the main components of unripe fruit oil. Estragole (61.08%), fenchone (23.46%), limonene + β-phellandrene + 1,8-cineole (8.68%), and α-pinene (1.15%) were the important components of ripe fruit oil.

Estragole, fenchone, and α-phellandrene were identified as the highest-level main constituents for flower oil. Sabinene, α-phellandrene, (*Z*)-β-ocimene, and γ-terpinene contents decreased with advanced vegetation, but fenchone, camphre, and estragole increased in all oils depending on vegetation times. While limonene + β-phellandrene + 1,8-cineole was established in unripe and ripe fruit oils, this compound was not established in flower oil. Germacrene D contents were decreased after the flowering period. All the oils consisted of monoterpenic hydrocarbons, oxygenated monoterpenes, and sesquiterpenes.

Table 1. Percentage Chemical Composition of Different Parts of Bitter Fennel (*F. vulgare*)

Compound	Retention time	Retention index	Fennel oil		
			Flower	Unripe fruit	Ripe fruit
α-Thujene	8.24	931	0.09	0.04	0.02
α-Pinene	8.48	939	0.96	1.52	1.15
Camphene	9.04	953	0.20	0.27	0.27
Sabinene	9.89	976	0.28	0.25	0.20
β-Pinene	10.03	980	0.12	0.13	0.07
Myrcene	10.54	991	0.93	1.19	0.95
α-Phellandrene	11.07	1,005	5.77	3.30	0.72
α-Terpinene	11.45	1,018	0.03	0.04	0.02
p-Cymene	11.73	1,026	3.26	—	—
Limonene + β-phellandrene	12.73	1,031	10.94	—	—
Limonene + β-phellandrene + 1,8-cineole	11.95	1,033	—	12.99	8.68
(*Z*)-β-Ocimene	12.17	1,040	0.35	0.25	0.10
(*E*)-β-Ocimene	12.51	1,050	0.03	0.02	—
γ-Terpinene	12.90	1,062	1.88	1.79	0.68
cis-Sabinene hydrate	13.22	1,068	0.04	0.03	—
cis-Limonene hydrate	13.39	1,071	0.07	0.12	0.06
Fenchone	14.04	1,087	13.53	19.18	23.46
2-Methylbutyrate, isoamyl	14.25	1,096	0.03	—	—
2-Methylbutyrate, 2-methylbutyl	14.38	1,100	0.08	—	—
Amyl isovalerate	14.39	1,100	—	0.08	0.10
Isovalerate	14.48	1,103	0.11	0.07	—
Isoamyl valerate	14.50	1,104	—	—	0.09
Fenchole	15.94	1112	0.02	—	—
Endofenchole	15.96	1,116	—	0.02	—
cis-p-Mentha-2,8,-dienol	16.05	1,120	0.05	—	—
cis-m-2-En-1-ol-pinane-2-ol	16.16	1,123	0.09	—	—
cis-m-2,8-Dien-1-ol	16.56	1,135	0.06	—	—
trans-Mentha-2-en-1-ol	16.76	1,141	0.04	—	—
Camphre	16.91	1,145	0.39	0.48	0.58
Neoisopulegole	17.30	1,156	0.02	—	—
Terpinene-4-ol	18.01	1,180	0.14	0.07	0.06
Estragole	18.97	1,208	53.08	56.11	61.08
Endofenchyl acetate	19.25	1,220	0.94	0.39	0.43
Exofenchyl acetate	19.68	1,233	3.27	—	0.43
α-Phellandrene epoxide	20.66	1,242	0.10	—	—
(*E*)-Anetole	21.25	1,283	—	0.01	—
Bornyl acetate	21.24	1,284	0.15	—	—
Germacrene D	26.90	1,480	0.28	0.05	—

A dash indicates the compound was not found.

A few reports on the essential oil of this species from different or similar origins have been published previously. The oil obtained from air-dried fruits of *F. vulgare* of Turkish origin contained methyl chavicol (47.09%), limonene (29.07%), fenchone (13.43%), fenchyl acetate (exo) (1.95%), *cis*-β-ocimene (1.41%), α-pinene (1.22%), and myrcene (1.08%) as the main constituents.[2] Specific estragole chemotypes are also known.[3] It was reported that the chemical composition of bitter fennel oils is very variable. The chemovarieties and the environmental conditions cause this variability. The major components from these were found to be methyl chavicol, *trans*-anethole, limonene, fenchone, γ-terpinene, and piperitonene oxide.[20]

Our results were generally similar to the literature findings with regard to components. Some variations may be due to the different climate factors and handling, collection, and ripening times. At the same time, these findings indicated that the oil of Turkish fennel (bitter) belongs to the methyl chavicol (estragole)-rich type.

Inhibitory effect of fennel oils

The inhibitory effects of flower, unripe fruit, and ripe fruit oils of fennel (bitter) (*F. vulgare* subsp. *piperitum*) against *A. alternata*, *F. oxysporum*, and *R. solani* are given in Table 2.

The oils exerted varying levels of antifungal effects on the growth of experimental fungi. The oils of 40 ppm lev-

TABLE 2. INHIBITORY ZONES OF FLOWER, UNRIPE FRUIT, AND RIPE FRUIT OF BITTER FENNEL AGAINST MYCELIAL GROWTH OF SOME FUNGI

Day, essential oil, concentration (ppm)	*Inhibitory zone (in mm) against fungus*		
	F. oxysporum	A. alternata	R. solani
3			
Flower			
10	24	20	25
40	–	–	–
Control	29	30	35
Unripe fruit			
10	22	17	20
40	–	–	7
Control	29	30	35
Ripe fruit			
10	19	18	24
40	–	–	–
Control	29	30	35
4			
Flower			
10	31	39	34
40	–	–	9
Control	36	43	47
Unripe fruit			
10	27	26	31
40	17	–	19
Control	36	43	47
Ripe fruit			
10	25	28	39
40	14	–	
Control	36	43	47
5			
Flower			
10	37	59	44
40	10	–	19
Control	42	59	64
Unripe fruit			
10	34	47	46
40	20	–	33
Control	42	59	64
Ripe fruit			
10	30	39	53
40	23	–	12
Control	42	59	64
6			
Flower			
10	40	67	60
40	19	–	30
Control	51	65	68
Unripe fruit			
10	41	56	57
40	28	–	45
Control	51	65	68
Ripe fruit			
10	35	50	63
40	29	–	22
Control	51	65	68
7			
Flower			
10	48	80	72
40	26	–	38
Control	56	71	75

(continued)

Table 2. Inhibitory Zones of Flower, Unripe Fruit, and Ripe Fruit of Bitter Fennel Against Mycelial Growth of Some Fungi (Cont'd)

Day, essential oil, concentration (ppm)	*Inhibitory zone (in mm) against fungus*		
	F. oxysporum	A. alternata	R. solani
Unripe fruit			
10	47	66	67
40	33	–	57
Control	56	71	75
Ripe fruit			
10	40	59	75
40	34	–	35
Control	56	71	75
8			
Flower			
10	58	85	80
40	53	–	50
Control	61	82	84
Unripe fruit			
10	52	74	77
40	40	–	65
Control	61	82	84
Ripe fruit			
10	47	70	84
40	40	–	47
Control	61	82	84
9			
Flower			
10	59	86	85
40	38	–	58
Control	67	86	87
Unripe fruit			
10	58	80	81
40	45	–	76
Control	67	86	87
Ripe fruit			
10	51	80	87
40	44	–	57
Control	67	86	87
10			
Flower			
10	67	87	88
40	45	–	69
Control	71	87	90
Unripe fruit			
10	64	84	86
40	50	–	82
Control	71	87	90
Ripe fruit			
10	58	85	90
40	52	–	66
Control	71	87	90
11			
Flower			
10	69	90	89
40	50	–	80
Control	75	90	90
Unripe fruit			
10	68	89	85
40	57	–	89
Control	75	90	90

TABLE 2. INHIBITORY ZONES OF FLOWER, UNRIPE FRUIT, AND RIPE FRUIT OF BITTER FENNEL AGAINST MYCELIAL GROWTH OF SOME FUNGI (CONT'D)

Day, essential oil, concentration (ppm)	*Inhibitory zone (in mm) against fungus*		
	F. oxysporum	A. alternata	R. solani
Ripe fruit			
10	62	89	90
40	57	–	79
Control	75	90	90
12			
Flower			
10	75	90	90
40	55	–	89
Control	77	90	90
Unripe fruit			
10	74	90	89
40	62	–	90
Control	77	90	90
Ripe fruit			
10	68	90	90
40	63	–	89
Control	77	90	90
13			
Flower			
10	78	90	90
40	61	–	90
Control	79	90	90
Unripe fruit			
10	78	90	90
40	67	–	90
Control	79	90	90
Ripe fruit			
10	72	90	90
40	69	–	90
Control	79	90	90
14			
Flower			
10	84	90	90
40	67	–	90
Control	82	82	90
Unripe fruit			
10	78	90	90
40	74	–	90
Control	82	90	90
Ripe fruit			
10	78	90	90
40	73	–	90
Control	82	90	90
15			
Flower			
10	84	90	90
40	70	–	90
Control	83	90	90
Unripe fruit			
10	79	90	90
40	77	–	90
Control	83	90	90
Ripe fruit			
10	80	90	90
40	77	–	90
Control	83	90	90

(continued)

TABLE 2. INHIBITORY ZONES OF FLOWER, UNRIPE FRUIT, AND RIPE FRUIT OF BITTER FENNEL AGAINST MYCELIAL GROWTH OF SOME FUNGI (CONT'D)

Day, essential oil, concentration (ppm)	*Inhibitory zone (in mm) against fungus*		
	F. oxysporum	A. alternata	R. solani
16			
Flower			
10	86	90	90
40	78	–	90
Control	84	90	90
Unripe fruit			
10	80	90	90
40	78	–	90
Control	84	90	90
Ripe fruit			
10	82	90	90
40	79	–	90
Control	84	90	90
17			
Flower			
10	86	90	90
40	83	–	90
Control	85	90	90
Unripe fruit			
10	80	90	90
40	80	–	90
Control	85	90	90
Ripe fruit			
10	83	90	90
40	79	–	90
Control	85	90	90
18			
Flower			
10	87	90	90
40	85	–	90
Control	86	90	90
Unripe fruit			
10	81	90	90
40	83	–	90
Control	86	90	90
Ripe fruit			
10	82	90	90
40	80	–	90
Control	86	90	90
19			
Flower			
10	90	90	90
40	86	–	90
Control	90	90	
Unripe fruit			
10	82	90	90
40	84	–	90
Control	90	90	90
Ripe fruit			
10	84	90	90
40	82	–	90
Control	90	90	90

A dash indicates 100% inhibition.

TABLE 3. ANTIFUNGAL EFFECT OF FENNEL (BITTER) OIL (PERCENT INHIBITION) AT 10 AND 40 PPM LEVELS IN THE CULTURE MEDIUM ON SOME FUNGI

		F. vulgare *subsp.* piperitum					
		Flowers		*Unripe fruit*		*Ripe fruit*	
Fungus	*Day*	*10 ppm*	*40 ppm*	*10 ppm*	*40 ppm*	*10 ppm*	*40 ppm*
F. oxysporum	3	17	100	24	100	34	100
	4	14	100	25	53	31	61
	5	12	76	19	52	29	45
	6	22	63	20	45	31	43
	7	14	54	16	41	29	39
	8	5	46	15	34	23	34
	9	12	43	13	33	24	34
	10	6	26	10	30	18	27
	11	8	33	9	24	17	24
	12	3	32	4	19	12	18
	13	1	23	1	15	9	13
	14	−2	18	5	10	5	11
	15	−1	16	5	7	4	7
	16	−2	7	5	7	2	6
	17	−1	2	6	6	2	7
	18	−1	1	6	3	5	7
	19	—	4	9	7	7	9
A. alternata	3	33	100	43	100	40	100
	4	9	100	40	100	35	100
	5	0	100	20	100	34	100
	6	−3	100	14	100	23	100
	7	−13	100	7	100	17	100
	8	−4	100	10	100	15	100
	9	—	100	7	100	7	100
	10	—	100	3	100	2	100
	11	—	100	1	100	1	100
	12	—	100	—	100	—	100
	13	—	100	—	100	—	100
	14	—	100	—	100	—	100
	15	—	100	—	100	—	100
	16	—	100	—	100	—	100
	17	—	100	—	100	—	100
	18	—	100	—	100	—	100
	19	—	100	—	100	—	100
R. solani	3	29	100	43	80	31	100
	4	28	81	34	60	17	100
	5	31	70	28	48	17	81
	6	12	56	16	34	7	68
	7	4	49	11	24	—	53
	8	5	40	8	23	—	44
	9	2	33	7	13	—	34
	10	2	23	4	9	—	27
	11	1	11	6	1	—	12
	12	—	1	1	—	—	1
	13	—	—	—	—	—	—
	14	—	—	—	—	—	—
	15	—	—	—	—	—	—
	16	—	—	—	—	—	—
	17	—	—	—	—	—	—
	18	—	—	—	—	—	—
	19	—	—	—	—	—	—

A dash indicates no inhibition.

els of fennel showed antifungal activity against mycelial growth of *A. alternata*, whereas 10 ppm levels were ineffective (Table 2). A 100% fungistatic effect was observed with 40 ppm doses of fennel oils. All oils showed a weak inhibitory effect according to the control until 11 days of incubation, while 40 ppm levels of oils showed a mostly stronger inhibitory effect than 0 ppm levels of oils on the growth of *R. solani*. The 10 ppm level of fennel flower oil stimulated mycelial growth of *F. oxysporum* after 13 days of incubation. Increasing levels of oil doses caused greater inhibition of the mycelial growth of all fungi (Table 3). Each dose of fennel oils exhibited a variable degree of fungitoxic activity against *F. oxysporum*. In addition, oils exhibited weak activity after 12 days of incubation against the same fungus. The results presented in Table 3 show that the fungus most affected by fennel oils was *A. alternata*, followed by *F. oxysporum*. The greatest inhibition percentage against *F. oxysporum*, at the 10 ppm dose, was 34%. The 10 and 40 ppm doses of unripe and ripe fruit oils exhibited moderate inhibition against *F. oxysporum* (Table 3). Consequently it is possible to say that the oil of the 40 ppm dose of fennel caused a complete inhibition (100%) of mycelial growth of *A. alternata*. The analyses show that fennel oils exhibited particular degrees of fungistatic activity depending on the doses.

Several studies have been conducted on the antimicrobial properties of herbs, spices, and their derivatives such as essential oils, extracts, and decoctions.[6,9,17,21–26] Some researchers have reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity.[7,27] Vigorous plant tissues contain many natural antifungal compounds, and these compounds defend plants against disease.[28] The inhibitory effects of phenolic compounds as eugenol, thymol, and cumin aldehyde compared with those of the terpenes carvone, borneol, and thujone were studied by Farag *et al*.[6] Some decrease of the inhibitory effect can probably be ascribed to evaporation from recooling of their essential oils during boiling. In addition to this, it is also known that the composition of decoctions and their antimicrobial effects depend on plant species and that their antimicrobial effects depend on plant species and regional conditions. It is well known that the phenolic components of essential oils show the strongest antimicrobial activity, followed by aldehydes, ketones, and alcohols.[29–31]

In conclusion, some of the oils used in this work have a partially effective inhibitory effect on the tested fungi. The results suggest the potential use of some hydrosols as antifungal preservatives in food. In food preparation and food processing such as storing, the use of high doses of fennel oils may provide a greater inhibitory effect on fungal growth. The fungistatic activity of spice oils should also prove to be a particularly interesting field for applications within the food, stored products, and cosmetic industry. Further research in this area has the potential to extend the usefulness of natural plant products and other biopesticides in crop production systems.

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