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Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage

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Abstract -The composition of the essential oil of *Pimpinella anisum* L fruit is determined by GC and GC-MS. The volatile oil content obtained by hydrodistillation was 1.91%. Ten compounds representing 98.3% of the oil was identified. The main constituents of the oil obtained from dried fruits were trans-anethole (93.9%) and estragole (2.4%). The olfactorially valuable constituents that were found with concentration higher than 0.06% were (E)-methyeugenol, α-cuparene, α-himachalene, β-bisabolene, p-anisaldehyde and cis-anethole. Also, the different concentrations of anise oil exerted varying levels of inhibitory effects on the mycelial growth of *Alternaria alternata, Aspergillus niger* and *Aspergillus parasiticus* used in experimental. The results showed that the most effected fungus from anise oil was *A. parasiticus*, which is followed by *A. niger* and *A. alternata*. Individual of this plant oil may provide a useful to achive adequate shelf-life of foods.

Key words: anise, *Pimpinella anisum*, essential oil, composition, trans-anethole, fungi, inhibitory effect.

INTRODUCTION

Anise (*Pimpinella anisum* L.), belonging to the Umbelliferae family is an annual herbaceous and a typical aromatic plant, which grows in several regions all over the world (Omidbaigi *et al*., 2003; Rodrigues *et al*., 2003; Askari *et al*., 2005). It is hardy to zone 8 and is frost tender. It is in leaf from May to October, in flower in July, and the fruits ripen from August to September. The aromatic seed is eaten raw or used as flavouring in raw or cooked foods such as soups, biscuits, confectionery, pies, bread and cakes (Hedrick, 1972; Riotte, 1978; Phillips and Foy, 1990). An essential oil from the seed is used as a food flavouring in fish, poultry, soups, root vegetable dishes, sweets, ice cream, chewing gum, pickles (Bown, 1995; Omidbaigi *et al*., 2003). It is also often used to flavour alcoholic drinks such aspernod, ouzo and anisette (Phillips and Foy, 1990; Bown, 1995). Anise seed is also used in some curries and seafood dishes, and is used as a breath sweetener and digestive aid (Anonymous, 2005a). It is also used in perfumes, toothpaste and the liquor industry (Dizdaroğlu and Balkan, 1996; Omidbaigi *et al*., 2003). The essential oil extracted from *P. anisum* fruit has antiseptic, antispasmodic, carminative, digestive, fungicide, asthma, chronic cough, expectorant, stimulant, stomachic, tonic. It may also have modest antiparasitic and antihelminthic actions and has been recommended by some practitioners to treat mild intestinal parasite infections (Baytop, 1984; Kubo *et al*., 1993; Chevallier, 1996; Singh *et al*., 1998; Omidbaigi *et al*., 2003).

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Spices, herbs and their derivatives are used in foods for their flavours and aroma (Dorman and Deans, 2000). The chemical composition of essential oil of several *Pimpinella* species has been studied (Embong *et al*., 1977; Ashraf *et al*., 1980; Ivanic *et al*., 1983; Lawrence, 1984; Başer and Özek, 1996; Omidbaigi *et al*., 2003; Rodrigues *et al*., 2003; Askari *et al*., 1998, 2005). There are usually considerably variations in the major components within this species: Embong *et al*. (1977) established (E)-anethole (72.2%), (Z)-anethole (1.1%), anisyl ceton (0.9%), β-caryophyllene (0.8%) and carvone (0.3%); Lawrence (1984) identified twenty-two components of the anise oil with the major components of (E)-anethole (85%), (Z)-anethole (2.2%) and methyl chavicol (1.02%); Askari *et al*. (1998) identified (E)-anethole (90%), eugenyl acetate (2%), γ-gurjunene (1.85%) and estragole (1.04%).

Recently, there has been considerable emphasis on studies involving essential oil and extracts of spices and their constituents for inhibiting the growth of microbes. Fungal infections are an important problem especially in medicine, and also in plant pathology. Infections caused by *Aspergillus* species are common in immunocompromised patients and carry significant treatment costs and mortality (Sokovic *et al*., 2002). It has been known for some time that certain crude drugs and spices contain substances with antifungal ability in their derivatives (Farag *et al*., 1989; Deans and Svoboda, 1990; Akgül *et al*., 1991; Özcan, 1998; Sokovic *et al*., 2002; Özcan, 2003; Boyraz and Özcan, 2006). The aim of this study was to determine the antifungal activity against *Alternaria alternata, Aspergillus niger* and *Aspergillus parasiticus* mycelial growth and chemical composition of anise seed (*P. anisum* L.) growing in Turkey.

MATERIAL AND METHODS

Plant material. Dried seeds of *Pimpinella anisum* L. were collected at the ripened stage in Burdur, Turkey in August 2004. The anise fruits were dried in the shade at room temperature. Plant of *Pimpinella anisum* L. was identified and authenticated by Dr Hüseyin Dural, plant taxonomist at the Biology Department of Selçuk University.

Recovery of the essential oil. Dried seeds of the plants (about 100 g) were ground into small pieces and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus; the oils obtained were dried over anhydrous sodium sulphate.

Identification of components. For the identification of components, analytical Hp 5890 gas chromatograph (GC, Shimadzu Corporation Kyoto, Japan) equipped with a DELSI 121 C apparatus, fitted with a flame ionisation detector and a CP WAX 51 fused silica column (25 m x 0.3 mm; 0.25 mm film thickness) was used. Temperature was kept at 50 °C for 5 min and programmed to reach 220 °C at the rate of 3 °C per min. A CP WAX 51 fused silica WCOT column (60 m x 0.3 mm) for Gas Chromotograph/Mass Spectrometer was used with helium as carrier gas. For GC/MS, a CPWAX 52 fused silica CB column (50 m x 0.25 mm) was used with helium as carrier gas (flow rate 1 ml/min) and coupled to a HP mass spectrometer (ionisation energy: 70 eV; ion source temperature and connection parts:180 °C). Temperature programming was from 50-240 °C at the rate 3 °C/min. Injector temperature was 240 °C. The components were identified by comparing linear Kovats indices (KI), their retention times and mass spectra with those obtained from the authentic samples and/or the Mass Spectra Library.

The percentage composition of the essential oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and mass spectra with corresponding data in the literature (Adams, 2001).

Microorganism. *Aspergillus parasiticus* NRRL 2999 was provided USDA, Agricultural Research Service, National Center for Agricultural Utilization Res. Service, Illinois, USA, *Aspergillus niger* and *Alternaria alternata* were obtained from the collections of Plant Protection Department, Faculty of Agriculture, Selcuk University.

Media. In order to obtain microorganism growth was used a standard agar medium, PDA: 200 g potato juice, 20 g D(+) glucose, 15 g agar-agar, 100 ml distilled water). For the determination of fungi toxic effect was used Czapex Dox Agar: 30 g sucrose, 3 g sodium nitrate, 0.5 g magnesium sulphate, 1 g potassium hydrogen phosphate, 13 g agar, 1000 ml distilled water.

Assessment of inhibition of fungal growth. The effects of essential oils of flowers of anise fruits were determined on *Alternaria alternata, Aspergillus parasiticus* and *Aspergillus niger* growth using Czapek-Dox agar medium. Discs of the test fungi (5 mm i.d.), cut from the periphery of 7-day-old cultures, were inoculated separately on to each assay plate and incubated at 25 °C for 7 days. Doses of each essential oil (10-100 ppm) were added on the sterile disc paper (10 mm i.d.), and put into Petri plates. The colony diameter was then measured and the percentage mycelial inhibition calculated following the equation:

$$I = C - T/C \times 100$$

where I is inhibition (%), C is the colony diameter of mycelium from a control Petri plate (mm) and T is the colony diameter of mycelium from a test Petri plate (mm) (Deans and Svoboda, 1990). Three replicated of each treatment were carried out and averages calculated. Control sets were run simultaneously, using the medium without any essential oils.

Statistical analysis. The data were subjected to ANOVA using randomised complete block design with statistical analyses system Anova procedure. (Püskülcü and İkiz, 1989).

RESULTS AND DISCUSSION

The oil yield from sample of anise fruits was 1.91%. The oil was colourless to pale-yellow in colour. *Pimpinella anisum* oil yield was compared with the oils of other *Pimpinella* ssp. The yield of seeds of *P. anisum* and *Pimpinella serbica* were 3.13-10.67% and 2.0-3.2%, respectively (Ivanic *et al*., 1983) and the yield of seeds of *Pimpinella aurea* was 2.0% (Askari *et al*., 2005).

The constituents identified in the anise (*P. anisum* L.) fruit oil are given in Table 1. Compounds are listed in order of their elution. Ten components were identified, accounting for 98.3%. The identification of the individual GC peaks was made by compared their retention times with those of the authentic samples and by matching the mass spectral (MS) data with those held in the Mass Spectra Library.

The results in Table 1 showed that the oil contained mainly phenylpropanoids and sesquiterpenoid hydrocarbons. The major constituents of the oil were trans-anethole (93.9%), estragole (2.4%) and γ-himachalene (1.1%). It is of interest to note the presence of trans-anethole (93.9%) in very high percentages, which was distinctive *P. anisum*.

The essential oil fraction of the *P. anisum* showed differences and similarities from many anise species growing in the several region of world, in regards to compounds, in the variety of its components and their relative quantity. The

TABLE 1 – Essential oil composition of anise seeds

KI*	Constituents	Percentage
1195	Estragole	2.4
1251	Cis-anethole	0.2
1252	p-anisaldehyde	0.1
1283	Trans-anethole	93.9
1400	(E)-methyleugenol	0.1
1447	α-himachalene	0.1
1479	γ-himachalene	1.1
1494	α-zingiberene	0.2
1506	α-cuparene	0.1
1506	β-bisabolene	0.1
Total		98.3

* Kovats indices.

main constituents of *P. aurea* D.C. oil were β-bisabolene (50.8%), viridifloral (37.0%), germacrene D (2.9%) and α-zingiberene (2.6%) (Askari *et al*., 2005). Askari *et al*. (1998) extracted 3.3% oil from the ripened fruits of anise and identified eleven components of it with the major components of (E)-anethole (90%), eugenyl acetate (2%), γ-gurjunene (1.85%) and estragole (1.04%).%). Embong *et al*. (1977) established (E)-anethole (72.2%), (Z)-anethole (1.1%), anisyl ceton (0.9%), β-caryophyllene (0.8%) and carvone (0.3%). Lawrence (1984) identified twenty-two components of the anise oil with the major components of (E)-anethole (85%), (Z)-anethole (2.2%) and methyl chavicol (1.02%). Ashraf *et al*. (1980) isolated 1.7% oil from anise fruit and identified six components of *Pimpinella aromatica* oil (Başer and Özek, 1996). The observed differences may be probably due to different environmental and species factors that can influence the oil composition.

The olfactorially valuable constituents that were found with concentration higher than 0.06% were (E)-methyeugenol, α-cuparene, α-himachalene, β-bisabolene, p-anisaldehyde and cis-anethole.

The oil of *P. anisum* presented various degrees of inhibition against the fungi (*Alternaria alternata, Aspergillus niger* and *Aspergillus parasiticus*) (Tables 2, 3). The results showed that the most effected fungus from anise oil was *A. parasiticus*, which is followed by *A. niger* and *A. alternata*.

TABLE 2 – Inhibitory effect of *Pimpinella anisum* oil

Days	Concentrations (ppm)	Inhibitory effect (mm)		
		Alternaria alternata	*Aspergillus niger*	*Aspergillus parasiticus*
2	Control (0)	28±2a*	41±2a	11±3a
	10	0**	38±4a	9±1a
	30	0	34±3b	8±2a
	50	0	28±5b	7±1b
	70	0	24±3b	6±1b
	100	0	25±3b	5±1b
3	Control (0)	33±3a	47±3a	17±2a
	10	20±3b	42±3a	15±2a
	30	14±1b	37±2b	13±1a
	50	15±1b	33±1b	11±1b
	70	17±2b	31±1b	9±1b
	100	19±3b	30±2b	8±2b
4	Control (0)	38±3a	54±6a	21±2a
	10	29±1b	48±5a	18±1a
	30	25±3b	43±5b	16±2b
	50	31±2b	42±4b	14±3b
	70	20±4b	42±6b	14±1b
	100	31±4b	51±7a	11±1c
5	Control (0)	44±5a	61±3a	32±4a
	10	37±1c	53±4a	30±1a
	30	33±3c	48±5b	26±3b
	50	42±2a	45±3b	23±4b
	70	22±1b	46±4b	19±1c
	100	35±3c	50±2a	17±2c
6	Control (0)	61±4a	69±7a	43±4a
	10	45±4b	57±5a	39±1a
	30	43±4b	53±5b	36±2b
	50	50±6b	52±4b	31±3b
	70	30±3c	51±3b	28±2b
	100	47±7b	52±6b	28±2b
7	Control (0)	67±3a	74±6a	46±6a
	10	50±6b	61±3b	41±2a
	30	45±4c	55±7b	37±3b
	50	55±3b	53±2b	33±2b
	70	37±2c	52±3b	32±1b
	100	58±2a	54±2b	32±1b
8	Control (0)	71±5a	77±2a	51±4a
	10	54±4b	68±4a	48±3a
	30	55±6b	61±6b	39±5b
	50	62±7a	57±7b	36±3b
	70	43±5c	55±3c	33±2b
	100	61±6a	53±3c	32±1b
9	Control (0)	78±7a	80±7a	57±4a
	10	59±3b	74±2a	49±6a
	30	59±4b	63±5b	43±5b
	50	68±4b	60±8b	41±7b
	70	50±2b	57±4c	37±4c
	100	71±6a	55±3c	34±3c

(continued)

TABLE 2 – (*Follow*) – Inhibitory effect of *Pimpinella anisum* oil

Days	Concentrations (ppm)	Inhibitory effect (mm)		
		Alternaria alternata	*Aspergillus niger*	*Aspergillus parasiticus*
10	Control (0)	80±7a	83±6a	63±9a
	10	67±3b	77±5a	56±5b
	30	68±4b	66±6b	54±3b
	50	71±5b	64±8b	46±7c
	70	56±2c	60±5b	43±8c
	100	78±6a	58±3b	38±1c
11	Control (0)	84±3a	86±5a	69±7a
	10	73±3b	81±5a	59±4b
	30	74±2b	70±4b	57±5b
	50	79±3a	65±3c	49±3c
	70	62±2c	64±7c	48±2c
	100	83±7a	63±8c	44±2c
12	Control (0)	86±4b	87±5a	74±3a
	10	78±6a	82±5a	67±5b
	30	79±7a	76±4b	63±3b
	50	83±8a	69±3b	58±6b
	70	65±4b	68±2b	53±8b
	100	84±6a	70±7b	52±3b
13	Control (0)	88±7a	88±5a	82±5a
	10	80±5a	84±4a	75±5b
	30	84±6a	79±8b	70±2b
	50	85±4a	73±9b	67±7b
	70	74±9b	73±7b	64±7b
	100	85±5a	72±6b	57±6b
14	Control (0)	89±5a	89±6a	86±7a
	10	81±4a	86±5a	79±4a
	30	85±3a	81±4a	73±5a
	50	87±2a	80±8a	71±8a
	70	81±3a	79±9a	67±9b
	100	87±4a	77±7a	64±6b
15	Control (0)	90±2a	90±8a	90±9a
	10	82±4a	88±7a	81±7b
	30	86±6a	84±6b	76±8b
	50	88±7a	83±4b	75±4b
	70	88±4a	81±5b	71±3c
	100	90±3a	80±9b	69±6c

* Data are means ± standard deviation, letters with similar alphabets within the day column are significantly at $p < 0.05$ level; ** No growth.

Each doses of anise oil exhibited variable degree of fungistatic activity against the tested fungi. Increasing levels of oil doses caused higher inhibition on the mycelial growth. The results showed that high doses of oil caused partly inhibition against mycelial growth of tested fungi. The analysis showed that anise oil exhibited certain degrees of fungistatic activity depending on the doses. There was statistical difference in numbers of inhibition zones ($p < 0.05$). Some researches reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity (Deans and Svoboda, 1990; Caccioni *et al.,* 1998; Özcan, 1998, 2003; Erkmen and Özcan, 2001; Sokovic *et al*., 2002). Vigorous are compounds defence plants against diseases (Fawcet and Spencer, 1970). Sokovic *et al*. (2002) assayed the antifungal effect of *Origanum onites, Satureja thymbra, Salvia fruticosa* and *Salvia pomifera* subsp. *calycina* oils agains 13 fungal species. The oils presented various degrees of inhibition against all the fungi investigated. Antimicrobial activity of *O. fruticosa* oil was tested using commercial strains of organisms known to be among the common pathogens in many tropical and internal diseases. The oil exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungal species (Aboutabl *et al*., 1995). The fungistatic action of these compounds was set at concentrations between 250 and 1000 ppm depending on fungal species. 1,8-Cineole and the monoterpene hydrocarbons, tested at concentration of 1000 ppm, showed limited or no fungistatic activity. The inhibitory effects of sixteen spice hydrosols (anise, basil, cumin, dill, Aegean sage, fennel (sweet), laurel, mint, oregano, pickling herb, rosemary, sage, savory, sea fennel, sumac and thyme (black)) on the mycelial growth of *Aspergillus parasiticus* NRRL 2999 strain were investigated *in vitro*. Of these, sumac had least effect on the mycelial growth of *A. parasiticus* (Özcan, 2005), there were inhibition zones between 10 and 100 ppm concentrations ($p < 0.05$). It has been well known that the phenolic components of essential oils show the strongest antimicrobial activity, followed by aldehydes, ketones and alcohols (Azzouz and Bullerman, 1982; Shelef, 1983; Deans and Svoboda, 1990). The results suggest the potential use of some oils as antifungal preservatives in food. In food preparation and food processing such as storing, the use of high doses of anise oil may provide a greater inhibitory effect on

TABLE 3 – Percentage inhibition of *Pimpinella anisum* oil on mycelial growth of some fungi

Days	Concentrations (ppm)	Inhibition (%)		
		Alternaria alternata	*Aspergillus niger*	*Aspergillus parasiticus*
2	10	100	7	18
	30	100	17	27
	50	100	32	36
	70	100	41	45
	100	100	39	55
3	10	28	11	12
	30	50	21	24
	50	46	30	35
	70	39	34	47
	100	32	36	53
4	10	24	11	14
	30	34	20	24
	50	18	22	33
	70	47	22	33
	100	18	24	48
5	10	16	13	6
	30	25	21	19
	50	5	26	28
	70	50	25	41
	100	20	18	47
6	10	26	17	9
	30	30	23	16
	50	18	25	28
	70	51	26	35
	100	23	25	35
7	10	25	18	11
	30	33	26	20
	50	18	28	28
	70	45	30	30
	100	13	27	30
8	10	24	12	6
	30	33	21	24
	50	13	26	29
	70	39	29	35
	100	14	31	37
9	10	24	8	14
	30	24	21	25
	50	13	25	28
	70	36	29	35
	100	10	31	40
10	10	16	7	11
	30	15	20	14
	50	11	23	27
	70	30	28	32
	100	3	30	40
11	10	13	6	14
	30	12	19	17
	50	6	24	29
	70	26	26	30
	100	1	27	36
12	10	9	6	9
	30	8	13	15
	50	3	21	22
	70	2	22	28
	100	2	20	30
13	10	10	5	9
	30	5	10	15
	50	3	17	18
	70	16	17	22
	100	3	18	30
14	10	10	3	8
	30	4	9	15
	50	2	10	17
	70	10	11	22
	100	2	13	26
15	10	9	2	10
	30	4	7	16
	50	2	8	17
	70	2	10	21
	100	0	11	23

fungal growth. Further studies in this area have the potential to extend the usefulness of natural plant products and other biopesticides in crop production systems.

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