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Dill (*Anethum graveolens* L.) and Parsley (*Petroselinum crispum* (Mill.) Fuss) from Estonia: Seasonal Differences in Essential Oil Composition

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Abstract. The essential oil content and composition of dill and parsley growing in summer and wintertime in Estonia were studied using the Clevenger distillation method for oil isolation and gas chromatography for identifying the extracts. Antimicrobial activity against several test microorganisms (Escherichia coli, Staphylococcus albus, Bacillus mesentericus and Aspergillus flavus) was studied using the zone-of-inhibition method. The essential oil yield of dried aromatic plants grown in wintertime was 0.24% of dry weight for parsley, and 0.56% for dill, and 0.29% and 0.65% for plants, grown in summer, respectively. Twenty-five (25) compounds were identified representing over 98% of the oil components of dill and dill seeds. The principal components of dill leaf oil were α-Phellandrene (47.7–62.5%), myristicin (1.7–28.2%), dill ether (0.9–14.8%), β-phellandrene (7.4–7.5%), and limonene (3.7–3.8%). Thirty-four (34) essential oil components were identified in parsley leaves (≥ 96%) with the major constituents myristicin (30.7–42.7%), β-phellandrene (21.8–35.9%), *p*-1,3,8-menthatriene (5.4–10.0%), and β-myrcene (4.5–8.7%). Essential oils from summer grown plants possessed higher antimicrobial activity against all studied microorganisms.

Key words: dill, essential oil composition, herbs, parsley, seasonal differences

INTRODUCTION

Steam-distilled plant essential oils have been widely used instead of ordinary culinary herbs not only for food flavouring but also for bactericidal, fungicidal, and medicinal applications and in fragrances (Bakkali et al., 2008).

Essential oils usually contain a variety of volatile compounds such as mono- and sesquiterpenes, phenol-derived aromatic and aliphatic components. The present work is dedicated to essential oil composition study of two of the most popular herbs in our region – dill (*Anethum graveolens* L.) and parsley (*Petroselinum crispum* (Mill.) Fuss) grown in summer and winter time.

Five common antimicrobial compounds, α-pinene, cineole, limonene, linalool and geranyl acetate, have been effective against food-borne pathogens Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Capmpylobacter jejuni (Chen et al., 2008; Sandasi et al., 2008). Oregano, savory and thyme, which have terpenes, carvacrol, p-cymene, and thymol, have demonstrated antifungal and antibacterial activity that has attracted attention recently because of their potential food safety applications (Bendahou et al., 2008; Naidu, 2000).

Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives (Shan et al., 2007). Some herbs used today in Estonia are valued for their antimicrobial activity. However, there is no reported data on the seasonal differences in essential oil composition.

The objectives of this study were (1) to determine essential oil composition in field grown and greenhouse produced dill and parsley, and (2) to establish the relationship between antimicrobial activity and total content of known antimicrobial compounds in essential oils of dill and parsley grown in summer and winter seasons. In addition, essential oils from dill seed were also studied, since the oil content produced in seeds is much higher compared to dill leaves (Callan et al., 2007).

MATERIALS AND METHODS

Plant material was commercially grown and harvested in Estonia. Summer dill and parsley were field grown and purchased from Kadarbiku Köögivili OÜ. Greenhouse-grown plant material was purchased from Grüne Fee AS. Herb material was dried at room temperature for four weeks, since this method does not significantly change the chemical composition of the essential oil (Orav et al., 2003).

Essential oils were prepared from dried plant material by Clevenger distillation (USP XXII, NFXVII, 1990) on a laboratory scale, and the yield of essential oils was calculated per dry weight. All the necessary dilutions for the further experiments were prepared with ethanol.

A Chrom 5 chromatograph with FID on two fused silica capillary columns with bonded stationary phases SPB-5 (30 m × 0.25 mm, Supelco) and SW-10 (30 m × 0.25 mm, Supelco) were used to carry out GC analysis. The film thickness of both stationary phases was 0.25 mkm. Helium with a split ratio of 1:150 and flow rate of 30–35 cm s^{-1} was applied as the carrier gas. The temperature program was from 50-250°C at 2°C/min; the injector temperature was 200°C. A Spectra-Physics SP 4100 computing integrator was used for data processing. The oil components were identified by comparing their retention indices (RI) on two columns with the RI values of reference standards, and our RI data bank.

The antimicrobial activity was determined on test microorganisms consisting of the laboratory strains of *Escherichia coli*, *Staphylococcus albus*, *Bacillus mesentericus*, and *Aspergillus flavus*. Stock cultures of the bacterial strains were maintained at 4°C on meat peptone agar prepared in laboratory. *A. flavus* was stored at the same temperature on agar medium with malt broth prepared in laboratory. Essential oils were screened for their ability to inhibit the growth of microorganisms using a standard zone-of-inhibition test (ZIT) on meat peptone agar for bacterial cultures and malt agar for fungal strains. Test volume for paper disc inoculation was 5 mkl of oil sample. The plates were incubated at 30°C for four days, the zones of inhibition were measured every 24 h. All experiments were performed in a minimum of three replicates.

RESULTS AND DISCUSSION

In our study on plant material grown in different seasons, the yield of essential oil fractions was slightly different for summer and winter plant material (Table 1). The total content of essential oils was generally higher in plants grown during the summer season. Dill seeds showed much higher essential oil content compared to the leaves.

Table 1. Essential oil content in dried plant material of dill and parsley.

Plant material	Moisture content, %	Essential oil yield, % of dry weight
Dill, summer	85	0.65
Dill, winter	93	0.56
Dill seeds	5	3.50
Parsley, summer	83	0.29
Parsley, winter	92	0.24

The essential oil fractions stored under nitrogen atmosphere were identified gas-chromatographically, and the results are presented in Table 2.

Principle components of dill herb oil were α-Phellandrene (47.7–62.5%), myristicin (1.7–28.2%), dill ether (0.9–14.8%), β-phellandrene (7.4–7.5%), and limonene (3.7–3.8%); α-Phellandrene and dill ether were the major compounds in dill leaves, but their content was much higher in summer dill. That may account for a more specific dill flavour and taste in comparison with its winter analog. Myristicin was the second characteristic component in winter dill. It has been shown for Cuban and Hungarian dill leaves that the harvesting time does significantly influence the carvone content in leaves (Pino et al., 1995; Simandi et al., 1996). In Estonia the harvesting time was before flower formation whereas the abovementioned authors used dill leaves during the flowering or seed formation period. Carvone (75.9%) was the most characteristic compound of Estonian dill seed oil which is the major reason why dill seeds are widely used for flavouring pickles.

Thirty-four (34) essential oil components were identified in parsley leaves. There were significant seasonal changes in the major constituents of parsley essential oils: myristicin and *p*-1,3,8-menthatriene content was higher in summer parsley (42.7% vs. 30.7% in winter parsley, and 10.0% vs. 5.4%, respectively). In contrast, two other major oil components were less abundant in summer samples, β-phellandrene (21.8% vs. 35.9% in winter parsley), and β-myrcene (4.5% vs. 8.7% in winter parsley).

Table 2. Essential oil constituents in dried plant material of dill and parsley.

Compound	Dill winter	Dill summer	Dill seeds	Parsley winter	Parsley summer
α-Thujene	0.12	0.34	*tr*	-	-
α-Pinene	0.60	1.76	0.02	0.46	1.49
Sabinene	0.03	0.14	-	0.12	0.14
β-Pinene	0.49	0.12	*tr*	0.31	0.90
β-Myrcene	0.53	0.77	0.10	**8.73**	**4.25**
α-Phellandrene	**47.74**	**62.49**	**0.66**	1.70	1.22
p-Cymene	0.66	3.14	0.20	0.19	0.35
Limonene	3.76	3.70	18.41	*tr*	1.97
β-Phellandrene	**7.40**	**7.48**	**0.13**	**35.88**	**21.83**
(E)-β-Ocimene	-	-	-	0.08	0.27
γ-Terpinene	1.75	0.05	*tr*	0.13	0.21
α-*p*-Dimethyl-phencone	0.13	0.29	*tr*	-	-
p-cymenene	-	-	-	*tr*	2.68
Phencone	0.38	0.12	-	-	-
Terpinolene	-	-	-	0.82	0.80
p-1,3,8- Menthatriene	-	-	-	**5.39**	**9.97**
Linalool	*tr*	0.07	0.14	0.03	0.04
(*Z*)-*p*-menth-2-en-1-ol	*tr*	0.08	0.09	-	-
Dill ether	**0.94**	**14.79**	**0.08**	-	-
(*Z*)-Dihydrocarvone	*tr*	0.10	0.42	-	-
(*E*)-Dihydrocarvone	0.06	0.08	2.54	-	-
(*E*)-Carveol	*tr*	*tr*	0.45	-	-
α-Terpineol	-	-	-	0.12	0.54
Estragol	-	-	-	*tr*	0.26
Carvone	**2.62**	**tr**	**75.92**	-	-
Bornyl acetate	-	-	-	0.11	0.25
(*E*,*E*)-Decadienal	-	-	-	-	2.65
α-Copaene	-	-	-	0.16	0.21
2,5-Dimethyl-*p*-cymene	-	-	-	0.20	0.66
2,5-Dimethoxy-*p*-cymene	-	-	-	3.16	0.90
(*E*)-β-Caryophyllene	-	-	-	-	0.31
β-Ionone	-	-	-	0.34	0.07
(*Z*)-Anethole	*tr*	0.39	*tr*	-	-
Epi-bicyclosesquiphellandrene	-	0.40	-	-	-
Germacrene D	0.42	-	-	1.04	0.12
α-Bergaptene	-	-	-	1.40	0.29
Myristicin	**28.15**	**1.67**	**0.02**	**30.67**	**42.65**
α-Cadinol	0.44	*tr*	*tr*	0.14	0.06
β-Selinene	-	-	-	0.20	-
α-Muurolene	-	-	-	0.10	0.65
Elemicin	-	-	-	2.46	0.15
Germacrene D-4-ol	-	-	-	-	0.24
δ-Cadinol	-	-	-	0.14	0.06
Apiole	0.62	0.05	0.03	1.76	0.11
Phthalide isomer*	1.26	*tr*	*tr*	0.16	*tr*
Total oil components (%)	98.10	98.03	99.21	96.00	96.61

tr = trace (<0.01%)

Plant material grown in winter and summer seasons showed very different antimicrobial properties as presented in Table 3.

Dill and parsley grown during the summer season contained essential oils with significantly higher antimicrobial properties. The most active constituents are expected to belong to the aromatic phenolic compounds with a wide spectrum of antimicrobial activity (Tajkarimi et al., 2010). However, it is possible that the activity of the main compounds is modulated by other minor constituents (Hoet et al., 2006). Myristicin, α-thujene, α-pinene, sabinene and other essential oil compounds are also reported to be responsible for the antibacterial and antifungal activity of spices and herbs (Tajkarimi et al., 2010).

Table 3. Results of zone-of-inhibition test (ZIT) with dill and parsley essential oils (diameter of inhibition zone in mm).

Test organism	Dill winter	Dill summer	Dill seeds	Parsley winter	Parsley summer
S. albus	8.0	26.0	11.0	9.0	20.0
B. mesentericus	No inhibition	25.0	15.0	No inhibition	10.5
E. coli	9.0	29.5	19.0	10.0	29.0
A. flavus	8.0	24.7	16.5	18.0	23.0

CONCLUSIONS

Summarised results of the experiments have shown that significant differences in plant essential oil content and composition exist depending on growth season. Based on higher content of compounds with antimicrobial activity a strong inhibitory effect of essential oils of dill and parsley grown in summer time could assist in preserving foods although different nutrient content of food may influence microbial resistance.

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