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Original Scientific Paper

Compositional characteristics of the essential oil of *Pimpinella anisum* and *Foeniculum vulgare* grown in Serbia

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ABSTRACT: The essential oils obtained by hydrodistillation from the mature fruits of *Pimpinella anisum* and *Foeniculum vulgare* were analyzed by GC-MS. The oil of aniseed was characterized by higher amounts of *trans*-anethole (96.80%) than fennel essential oil (83.43%). The isomer of *trans*-anethole, methyl chavicol (estragol) was also present in both plants, with 1.36% in fennel and 0.19% in aniseed. Apart from these two phenylpropanoids, nine sesquiterpenes and one monoterpene were present in essential oil of *Pimpinella anisum*, which constituted the other 3.01% of essential oil, but only γ-himachalene was present with more than 1%. In the case of fennel, eight monoterpenes constituted the other 15.21% of essential oil, and limonene and fenchone were present with contributions of 9.34 and 4.58%, respectively.

Key words: aniseed, fennel, *trans*-anethole, methyl chavicol

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INTRODUCTION

Aniseed (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgare* Mill.) belongs to the *Apiaceae* family. This family is well known for its distinctive flavors, which come from essential oils, which are a mix of volatile fragrant compounds that give the essence of the plant. In aniseed there is around 1.5-5.0% essential oil (Ullah & Honermeier 2013), and in fennel seeds there is usually between 2-6% (Najdoska *et al.* 2010).

The phytotherapeutic applications of aniseed and fennel include stimulation of the digestive system, as appetizers, by increasing the digestibility of nutrients through increasing the secretion of digestible enzymes and developing hepatic activity (Mohammed & Abbas 2009, Areej *et al.* 2012). They are also often used for treatment of respiratory diseases (Esquivel-Ferriño et al. 2012**,** Sleman *et al.* 2013**)**. Apart from this, the essential oil of these plants possesses antimicrobial and antioxidant activity (Gülçin *et al.* 2003, Gende *et al.* 2009, Shahat *et al.* 2011), and other medicinal benefits.

The dominant constituent in essential oils of aniseed and fennel is *trans*-anethole, which has a sweet herbaceous odour and sweet taste. This comprises around 77-94% in aniseed (Orav *et al.* 2008), and 35–82% in fennel (Raal *et al.* 2012b). *Trans*-anethole is also present in high amount (72-92%) in *Illicium verum* Hook. f. (Ohira *et al.* 2009) which belongs to the *Magnoliaceae* family. Apart from natural sources, anethole is often produced by chemical synthesis.

Trans-anethole can mask unpleasant odours, so it is widely used as a masking agent in commodities, such as toilet soap, toothpaste, mouthwash, as well as a flavouring additive and scent in food industry products such as beverages, liquor, candy, baked goods, chewing gum and cigarettes. Anethole is also used in industrial products,

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deodorisation, medicine, feed additives, synthetic flavours, pesticides and so forth (Zongliang 2012).

The aim of this study was to determine the chemical composition of the essential oils obtained from seeds of *Pimpinella anisum* and *Foeniculum vulgare* from North Serbia, by GC-MS analysis.

MATERIAL AND METHOD

Plant material: Plants of *Pimpinella anisum* and *Foeniculum vulgare* were grown during 2012, in the North part of Serbia, known as Vojvodina Province (research state Mošorin - 45°18’ N, 20°09’ E, and altitude 111 m).

Isolation of the essential oil: Essential oil obtained by hydrodistillation using Clevenger-type apparatus from crushed mature fruits. The content of essential oil in fruits of *Pimpinella anisum* was 3.91%, and in *Foeniculum vulgare* 7.1%.

GC-MS analysis: The oil quality was assessed through analysis by combined gas chromatography and mass spectrometry. GC-MS analysis was performed by using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD), in positive ion electron impact (EI) mode. The separation was achieved by using Agilent 19091S-433 HP-5MS fused silica capillary column, 30m x 0.25mm i.d., 0.25 μm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 4.3 °C/min. Helium was used as carrier gas; inlet pressure was 25 kPa; linear velocity was 1ml/min at 210 °C. Injector temperature: 250 °C; injection mode: splitless. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; energy, 70 eV; mass scan range, 40-350 amu.

Identification of components was done on the basis of retention index (RI), their retention times (RT) and by comparison with reference spectra (Wiley and NIST databases).

RESULTS AND DISCUSSION

These plants grow well in Serbia, and have optimal agroecological conditions for high productivity and good quality in our country (Aćimović *et al.* 2013a,b). In this investigation, the content of the essential oils in mature fruits of aniseed and fennel was high, 3.91% and 7.1%, respectively.

Chromatograms of essential oils from the two species are shown in figures 1 and 2. The peak of *trans*-anethole in the case of aniseed (Fig 1) was at retention time 19.652 min, and in the case of fennel 19.392 min (Fig 2). This compound was identified as the most abundant in both essential oils.

A detailed description of all of the components detected by GC-MS found in the essential oils of the two plant species is shown in Table 1, which include the RI values of each component.

Twelve components were detected in aniseed, and ten in fennel essential oil, which constituted more than 99% of the total compounds. Only the isomers methyl chavicol and *trans*-anethole were present in both plants. Methyl chavicol is produced exclusively through transformation (isomerization) of *trans*-anethole, but the reverse situation is also possible (Molino 2000).

These two components belong to the class phenylpropenes. This class was most abundant in both plants. Aside from phenylpropenes, nine sesquiterpenes (2.39%) and one monoterpene (0.26%) were present in the essential oil of aniseed. In the case of fennel, eight monoterpenes constituted the remaining essential oil components (15.04%).

In essential oil of *Pimpinella anisum*, the most abundant component was *trans*-anethole with 96.8%, then γ-himachalene with 1.84%, while five components were present at less than 1%. These were: *trans*-pseudoisoeugenyl 2-methylbutyrate (0.32%), *cis*-dihydrocarvone (0.26%), methyl chavicol (0.19%), α-himachalene (0.13%) and β-himachalene (0.11%). Components found in traces

Figure 1. Chromatographic profiles of *Pimpinella anisum* essential oil

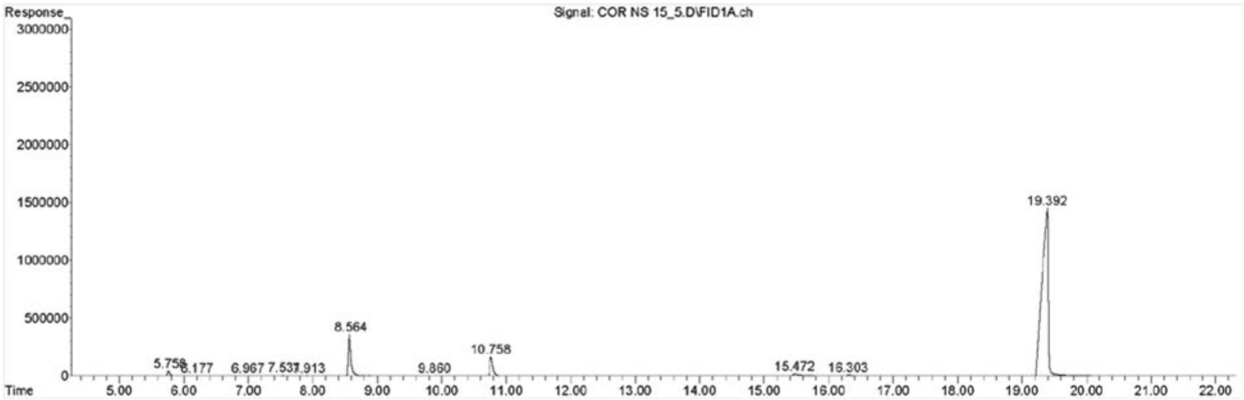

Figure 2. Chromatographic profiles of *Foeniculum vulgare* essential oil

Table 1. Components of essential oils of *Pimpinella anisum* and *Foeniculum vulgare*

Component	RI	*Pimpinella anisum*	*Foeniculum vulgare*
α-pinene	921	0	0.94
camphene	941	0	trace
sabinene	968	0	trace
myrcene	981	0	0.19
α-phellandrene	1005	0	trace
limonene	1021	0	9.34
γ-terpinene	1048	0	trace
fenchone	1078	0	4.58
cis-dihydrocarvone	1192	0.26	0.00
methyl chavicol	1200	0.19	1.36
trans-anethole	1294	96.80	83.43
cis-anethole	1255	trace	0
β-elemene	1335	trace	0
α-himachalene	1452	0.13	0
γ-himachalene	1482	1.84	0
trans-muurola-4(14),5-diene	1485	trace	0
UI	1487	trace	0
α-zingiberene	1499	trace	0
β-himachalene	1504	0.11	0
trans-pseudoisoeugenyl 2-methylbutyrate	1848	0.32	0
Monoterpenes		0.26	15.04
Phenylpropanoids		97.00	84.79
Sesquiterpenes		2.39	0.00
Total identified		**99.65**	**99.84**

UI-unidentified compound, **trace**-component present less than 0.1%

were: *cis*-anethole, *β*-elemene, *trans*-muurola-4(14),5-diene, *α*-zingiberene and one unidentified compound.

In essential oil of *Foeniculum vulgare*, *trans*-anethole was dominant with 83.43%, then limonene (9.34%), fenchone (4.58%) and methyl chavicol (1.36%). This last component had two peaks in the GC/MS analysis, the first at 15.472 and the second at 16.303 min. In fennel essential oil, two components were present at less than 1% (*α*-pinene and myrcene), and components found in trace amounts were camphene, sabinene, *α*-phellandrene and *γ*-terpinene.

Gende *et al.* (2009) found that the oils of *P. anisum* and *F. vulgare* were especially rich in *trans*-anethole, 96.3% and 92.7%, respectively. In our investigation, the concentration of *trans*-anethole in fruits of aniseed was almost same as in the Gende *et al.* (2009) study, while the fruits of fennel contained significantly less *trans*-anethole in our study in comparison with Gende *et al.* (2009). This is probably the effect of variety used in the study. We used sweet fennel *Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce* (Mill.). In another study on this variety of fennel, Telci *et al.* (2009) claimed that the content of *trans*-anethole in mature fruits was from 84.42 to 87.85% depending on umbel position. These authors claimed that the essential oil composition also depended on internal and external factors affecting the plant such as genetic structures and ecological conditions. Agricultural practices also have critical effects on yield and oil composition in essential oil crops, although essential oil has some main components that can vary significantly according to the maturation period.

In aniseed essential oil, the other principal compounds were *γ*-himachalene, then *trans*-pseudoisoeugenyl 2-methylbutyrate. In a study by Orav *et al.* (2008), the major component of essential oil from *Pimpinella anisum* L. fruits obtained from different geographical areas of Europe, was *trans*-anethole, and the other principal compound was also *γ*-himachalene in concentrations from 0.4 to 8.2%, and *trans*-pseudoisoeugenyl 2-methylbutyrate (0.4-6.4%). This was similar to our results. In addition, these authors emphasised that pseudoisoeugenyl 2-methylbutyrate is a typical component of the genus Pimpinella and is used as a phytochemical marker for this genus.

Cis–dihydrocarvone in our study was present at 0.26%. This component is usually present in other plants from the Apiaceae family, such as caraway (Raal *et al.* 2012a) and dill (Charles *et al.* 1995, Krüger & Hammer 1996). However, so far, it has not been detected in aniseed.

Our study showed that the second most abundant component in fennel is limonene, then fenchone. In a study conducted in Italy (Piccaglia & Marotti 2001) on 13 wild types of fennel, limonene content varied between 1.74 and 22.78%, and fenchone from 2.21 to 11.22%. These authors noted that the latitude of origin of localities seemed to influence the oil compositions. In this study, 21 components were determined, which was more than in our study (only 10), but all components which we detected were present in the Italian samples.

From hydrodistilled wild fennel fruits sampled in Montenegro, apart from ten components which were the same as in our experiment, *α*-thujone, *p*-cymene, 1,8 cineole, *β*-ocimene, camphor, *cis*-anethole and germacrene were determined (Damjanović *et al.* 2005). Low concentrations of α-phellandrene and γ-terpinene, as in our experiment, were also found in seeds of Turkish fennel by Guillén & Manzanos (1996). Compounds like α-pinene, camphene and myrcene were also found in fennel from Algeria (Zoubiri *et al.* 2010).

CONCLUSION

The findings of this study show that the essential oils from aniseed and sweet fennel grown in the North part of Serbia were characterized by high amounts of *trans*-anethole, 96.80% and 83.43%, respectively. The isomer of *trans*-anethole, methyl chavicol was also present in both plants, but in small amounts (in aniseed only at 0.19%, and in fennel at 1.36%). Considering that the amount of the essential oil was high, plants grown in this area represent a high quality raw material.

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REZIME

Hemijski sastav etarskih ulja *Pimpinella anisum* i *Foeniculum vulgare* gajenih u Srbiji

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Etarsko ulje dobijeno destilacijom vodenom parom iz zrelih plodova *Pimpinella anisum* i *Foeniculum vulgare* analizirano je GC-MS metodom. Etarsko ulje anisa se karakteriše većim sadržajem *trans*-anetola (96.80%), nego etarsko ulje komorača (83.43%). Isomer *trans*-anetola, metil kavikol (estragol) je takođe prisutan kod obe biljke, sa 1.36% kod komorača i 0.19% kod anisa. Pored ova dva fenilpropanoida, devet seskviterpena i jedan monoterpen je prisutan u etarskom ulju *Pimpinella anisum*, i čine ostatak od 3.01% etarskog ulja, ali je samo γ-himachalen prisutan u količini većoj od 1%. U slučaju komorača, osam monoterpena čine 15.21% etarskog ulja, a limonen i fenchon su prisutni sa 9.34 i 4.58%.

Ključne reči: anis, komorač, *trans*-anetol, metil kavikol