

This article was downloaded by: [Aston University]

On: 30 January 2014, At: 01:31

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gnpl20>

Essential oil composition of *Pimpinella anisum* L. fruits from various European countries

Anne Orav^a, Ain Raal^b & ELMAr Arak^b

^a Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

^b Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

Published online: 13 Aug 2008.

To cite this article: Anne Orav, Ain Raal & ELMAr Arak (2008) Essential oil composition of *Pimpinella anisum* L. fruits from various European countries, *Natural Product Research: Formerly Natural Product Letters*, 22:3, 227-232, DOI: [10.1080/14786410701424667](https://doi.org/10.1080/14786410701424667)

To link to this article: <http://dx.doi.org/10.1080/14786410701424667>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &

Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

Essential oil composition of *Pimpinella anisum* L. fruits from various European countries

ANNE ORAV†, AIN RAAL*‡ and ELMAR ARAK‡

†Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

‡Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

(Received 2 March 2007; in final form 2 May 2007)

Variations in the essential oil composition of *Pimpinella anisum* L. fruits obtained from different geographical areas of Europe were determined using capillary GC and GC–MS techniques. The essential oil content of the samples was 10.0–53.6 mL kg⁻¹ and did not conform to the European Pharmacopoeia standard in 5 samples out of 14. A total of 21 compounds were identified and significant quantitative differences were observed among the samples. The major component was *trans*-anethole (76.9–93.7%); the other principal compounds in oils were γ -himachalene (0.4–8.2%), *trans*-pseudoisoeugenyl 2-methylbutyrate (0.4–6.4%), *p*-anisaldehyde (*tr*-5.4%) and methylchavicol (0.5–2.3%). The highest content of *trans*-anethole (>90%) was found in the samples from Greece, Hungary, Scotland, Lithuania, Italy, and Germany (2 samples). Essential oil of aniseed from Estonia was rich in γ -himachalene (8.2%) and *trans*-pseudoisoeugenyl 2-methylbutyrate (6.4%). The sample from France contained the highest amount of anisaldehyde (5.4%) comparing with other samples (0–3.1%). β -Bourbonene and α -farnesene are determined in anise oil for the first time.

Keywords: *Pimpinella anisum* L.; Essential oil; European Pharmacopoeia; *trans*-Anethole; γ -Himachalene; *trans*-Pseudoisoeugenyl 2-methylbutyrate

1. Introduction

Anise, botanical name *Pimpinella anisum* L., plant family name Apiaceae (also known as Umbelliferae), is a dainty herbaceous annual plant with white flowers. Aniseed is endemic (indigenous) to Asia Minor, Egypt, and Greece, and is grown in Europe (Spain, Italy, Germany, Southern Russia) and South America. It is cultivated in Turkey, Russia, South Africa, Latin America, and Brazil. Anise fruits, which are commercially called “seeds”, are used as flavorings and crude drug. The essential oil from anise fruits is used in food processing, perfumes, toothpaste, and also in medicine. Anise root oil is also used [1–3].

Aniseed (*Anisi fructus*) belongs to European Pharmacopoeia (EP) and consists of the whole dry cremocarp of *P. anisum* L., which contains not less than 20 mL kg⁻¹ of essential oil [4]. The French Pharmacopoeia defines aniseed oil as obtained from the

*Corresponding author. Tel.: +372 7 375281. Fax: +372 7 375289. Email: ain.raal@ut.ee

ripe and dried fruits of *P. anisum* L. or *Illicium verum* Hook. fil. because the composition of the two essential oils are very similar [5].

The aroma of the anise essential oil (up to 60 mL kg^{-1} in the fruits) is dominated by *trans*-anethole, which is the character-impact compound and present in concentrations of 75–95%. Additional components are methylchavicol, *p*-anisaldehyde, γ -himachalene, zingiberene, and pseudoisoeugenyl 2-methylbutanoates and epoxypseudoisoeugenyl 2-methylbutanoates [1,6–24].

C_{12} sesquiterpenes (geijerene, pregeijerene) and phenylpropanoids (pseudoisoeugenyl 2-methylbutyrate, epoxypseudoisoeugenyl 2-methylbutyrate) are characteristic to the genus *Pimpinella* and are phytochemical markers for this genus [24–26]. Because *cis*-anethole has toxic properties, international laws limit the concentration of this compound in isolated natural anethole to 0.2% [1]. All oxidation products of anethole (anisic alcohol, anisaldehyde, anisic acid, anethol epoxide, *p*-methoxyphenylacetone, *p*-methoxypropiophenone, anisyl alcohol, and ketone) may be present in the anise oils. The presence of these compounds can be an indication of the deterioration of the oil [1,5].

Anethole is spasmolytic and stimulates respiratory secretions and expectoration. It also has mild estrogenic, local anesthetic, anticarcinogenic, and cytotoxic activity [5,20]. Aniseed is traditionally used for the symptomatic treatment of gastrointestinal disturbances and catarrh of upper respiratory tract [3,5,20].

The essential oil of anise fruits cultivated in Estonia has not been analyzed by capillary gas chromatography before. The objective of this work is to compare the composition of the essential oil from anise fruits cultivated in Estonia and in other European countries.

2. Results and discussion

Essential oil yields of *P. anisum* L. fruits obtained from different European countries ranged from 10.0 to 53.6 mL kg^{-1} (table 1). Thus differences between minimum and maximum contents are more than five times. The oil yield was surprisingly highest in aniseeds from Estonia harvested in 1999. The minimum limit of EP (20 mL kg^{-1}) did not exceed in 5 samples out of 14 (France, Russia 1 and 2, Scotland, Germany 1). The quality of the drug of aniseed seems to be a problem in Brazil also, where 71% of commercial samples of anise contain lower content of volatile compounds [27]. Thereafter we should take into consideration the established fact that the concentration of essential oil decreases rapidly in storage [5].

Essential oils were analyzed by capillary GC using two columns with different polarity and by GC–MS. Twenty-one compounds were identified in anise fruit oils constituting 96.3–99.6% of the total oil. The main compounds are listed in table 1 with their percentage range, mean%, and SEs mean. β -Bourbonene and α -farnesene are not mentioned before in literature as constituents of anise oil.

The major constituent of the essential oil from anise fruits were found to be *trans*-anethole (76.9–93.7%) (table 1). The other constituents with mean value over 1% were methylchavicol (0.5–2.3%), anisaldehyde (0–5.4%), γ -himachalene (0.4–8.2%), and pseudoisoeugenyl 2-methylbutyrate (0.4–6.4%). Mean percentage of the remaining constituents was below 1%. The minor constituents also found were nonanal (0–1.4%, earlier reported in [14]), isogeijerene (0–0.9% [16,24]), *cis*-anethole

Table 1. Content (%) of the principal components of the essential oil and essential oil yield (mL kg⁻¹) from *P. anisum* L. seeds from various European countries.

Sample origin	Methylchavicol	<i>p</i> -Anisaldehyde	<i>trans</i> -Anethole	γ -Himachalene	<i>trans</i> -Pseudoisoeugenyl 2-methylbutyrate	Oil yield (mL kg ⁻¹)
France	1.5	5.4	76.9	3.1	5.7	10.0 ^a
Hungary	0.5	0.2	91.3	3.9	1.0	26.1
Russia 1	0.5	0.6	85.7	5.6	1.8	14.8 ^a
Russia 2	0.8	0.8	85.1	4.1	2.0	11.3 ^a
Russia 3	1.5	1.8	87.7	2.5	1.7	23.1
Greece	1.3	2.8	90.2	2.6	0.9	48.3
Scotland	1.9	0.7	93.6	1.4	0.4	13.1 ^a
Lithuania	2.0	0.7	93.0	1.5	0.7	23.0
Spain	1.4	2.2	87.9	2.3	2.3	25.4
Italy	1.3	tr	92.2	2.2	1.5	20.4
Germany 1	2.0	tr	92.7	1.4	1.4	12.1 ^a
Germany 2	2.3	1.0	93.7	0.4	1.3	25.3
Czech	0.9	3.1	88.4	0.7	2.9	23.3
Estonia	0.6	0.1	78.1	8.2	6.4	53.6
Mean (<i>n</i> = 14)	1.32	1.38	88.32	2.85	2.14	23.6
SE mean	0.16	0.41	1.44	0.56	0.48	3.46
RI (SPB-5)	1197	1256	1285	1474	1841	
RI (NB-20M)	1670	1630	1820	1705	2567	
Earlier reported	[1,8-9,12-14,17-19,22,24]	[1,11,14,18-19,24]	[1,6-15,18-24]	[12,13,16-19,24]	[1,7,14,15,19,24]	

^aThe minimum limit of EP is not exceeded. Boldface designates the highest concentrations.

(0–2.0% [1,8,9,14,24]), pregeijerene (0–0.5% [10,13–15]), β -bourbonene (0–0.9% [not known before]), α -himachalene (0–0.4% [12,13,16,18,24]), β -farnesene (0–0.7% [13,24]), germacrene D (0–1.1% [13,18,24]), α -curcumene (0–0.4% [6,14,16,17,24]), zingiberene (0–1.1% [10,13–15,17,24]), α -farnesene (0–0.4% [not known before]), β -bisabolene (0–0.6% [13–15]), γ -cadinene (0–0.3% [24]), β -sesquiphellandrene (0–0.4 [14,16,24]), δ -cadinene (0–0.3 [24]), and epoxypseudoisoeugenyl 2-methylbutyrate (0–2.3% [10,15,24]).

The highest concentrations of *trans*-anethole (>90%) were found in the oils from Hungary, Greece, Scotland, Lithuania, Italy, and Germany. The most sesquiterpene-rich oil was obtained from Estonia (11.0%) and from Russia (7.7–7.9%). The samples from Estonia and France were characterized by high content of a *trans*-pseudoisoeugenyl 2-methylbutyrate (6.4 and 5.7%, accordingly). In the other samples, content of this compound was below 3%. Anisaldehyde (5.4%) rich oil was isolated from aniseed of France. The samples from Greece, Russia 3, Spain, Germany 2, and Czech contained this oxidation product 1–3%.

The essential oil of anise fruits cultivated in Estonia can be characterized by relative low percentage of *trans*-anethole (78.1%), but high content of γ -himachalene (8.2%) and *trans*-pseudoisoeugenyl 2-methylbutyrate (6.4%) compared with other samples studied.

By the EP [4] the commercial anise oil (*Anisi aetheroleum*) should contain linalool <1.5%, estragole 0.5–5%, α -terpineol <1.2%, *cis*-anethole 0.1–0.4%, *trans*-anethole 87–94%, anisaldehyde 0.1–1.4%, and pseudoisoeugenyl 2-methylbutyrate 0.3–2%. So, the content of methylchavicol (estragole) in the samples studied varied from 0.5 to 2.3%. Literature data [1,11,14,18,19,22,25] gave this value up to 5%, or even up to 10% [28]. The EP limit of methylchavicol in essential oils (0.5–5%) [4] was not exceeded in investigated samples. Estragole has shown mutagenic potential in various Ames tests [20]. The essential oils of samples from Russia, Spain, and Czech contain more *cis*-anethole (respectively 2.0, 0.8, and 0.7%) than allowed by the EP [4]. The content of anisaldehyde exceeded in 5 samples out of 14. In other 9 samples, its concentration was less than 1%. In the samples from France, Spain, Czech, and Estonia also the content exceeded the EP maximum limit of *trans*-pseudoisoeugenyl 2-methylbutyrate. However, these results do not emphatically indicate the low quality of analyzed anise samples. In [29], different isolation methods for the volatile components of anise are compared in terms of the difference of components obtained with each analytical procedure. Authors conclude that different isolation techniques result in compositionally different isolates. In our investigation, only the method of hydrodistillation by EP [4] was used to analyze all the 14 samples.

3. Experimental

3.1. Plant materials

Plant materials (commercial *P. anisum* L.) fruits were obtained from retail pharmacies from different European countries (total 14 samples) in 1999–2005: from France (Strasbourg, 1999), Hungary (Budapest, company Fitopharma kft., 2001), Russia 1 (Krasnogorsk, 2002), Russia 2 (Ural, company Fito-M, 2003), Russia 3 (Moscow, company Ivan-Tshai, 2004), Greece (Athens, company Καλογερογιαννης

Χρηστος, 2002), Scotland (Glasgow, company ScotMed Herbs, 2003), Lithuania (Kaunas, company Camelia, 2004), Spain (Barcelona, company Acofar, 2004), Italy (Vasto, 2004), Germany 1 (Nuremberg, St. Martins-Apotheke, 2004), Germany 2 (cultivated in Germany, obtained from Förde-Apotheka in Sweden, 2005), and from Czech (Prague, company Herbata, 2005). In Estonia, the fruits of aniseed are cultivated in Põltsamaa (Central Estonia, 1999). Voucher specimens (without number) have been deposited at the Botanical Museum, University of Tartu, Estonia [4].

3.2. Isolation of essential oil

The essential oil was isolated from dried drug of anise fruits by the distillation method described in the EP using 10 g of crushed drug, a 250 mL round-bottomed flask, and 100 mL water as the distillation liquid. 0.5 mL of xylene in the graduated tube was added to take up the essential oil. The distillation time was 2 h at a rate of 2.5–3.5 mL/min⁻¹. The drug should be reduced to a coarse powder and immediately used.

3.3. Capillary gas chromatography

Gas chromatography analysis was carried out using 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 NB-20M (50 m × 0.20 mm, NORDION, Finland). Film thickness of both stationary phases was 0.25 μm. Carrier gas helium with split ratio 1:150 and the flow rate 20–25 cm sec⁻¹ was applied. The temperature program was from 50–250°C at 2°C min⁻¹, the injector temperature was 200°C. A Hewlett–Packard Model 3390A integrator was used for data processing.

The identification of the oil components was accomplished by comparing their retention indices (RI) on two columns with the RI values of reference standards, our RI data bank and with literature data. The results obtained were confirmed by GC–MS.

The percentage composition of the oils was calculated in peak areas using normalization method without correction factors. The relative SD of percentages of oil components of three repeated GC analyses of single oil sample did not exceed 5%.

3.4. GC–MS analysis

The MS analysis was carried out on a Hewlett–Packard HP 5988 mass spectrometer and HP 5890A gas chromatograph using a DB-5 poly(5%-diphenyl-95%-dimethyl)-siloxane (25 m × 0.25 mm, film thickness 0.25 μm) fused-silica capillary column from J&W Scientific (USA). The column temperature was 50–280°C at 8°C min⁻¹.

Acknowledgement

Financial support for the work reported here was provided by the Estonian Science Foundation (Grant No. 4332).

References

- [1] M.A. Boelens. Spices and condiments II. In *Volatile Compounds in Food and Beverages*, H. Maarse (Ed.), pp. 449–482, Marcel Dekker, Inc., New York, Basel, Hong Kong (1991).
- [2] G. Samuelsson. *Drugs of Natural Origin*, Swedish Pharmaceutical Press, Stockholm (1992).
- [3] J. Barnes, L.A. Anderson, J.D. Phillipson. *Herbal Medicines: A Guide for Healthcare Professionals*, Pharmaceutical Press, London, Chicago (2002).
- [4] *European Pharmacopoeia*, 5th Edn, Vol. 2, Council of Europe, Strasbourg (2005).
- [5] J. Bruneton. *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2nd Edn, Intercept Ltd, Paris, New York (1999).
- [6] K.-H. Kubeczka, F. von Massow, V. Formacek, M.A.R. Smith. *Naturforsch.*, **B31**, 283 (1976).
- [7] G.T. Carter, H.K. Schnoes, E.P. Lichtenstein. *Phytochem.*, **16**, 615 (1977).
- [8] M.B. Embong, D. Hadziyev, S. Molnar. *Can. J. Plant Sci.*, **57**, 681 (1977).
- [9] M. Ashraf, M.A. Siddiqui, M.K. Bhatti. *Pakistan J. Sci. Ind. Res.*, **23**, 211 (1980).
- [10] K.-H. Kubeczka, I. Ullman. *Biochem. Syst. Ecol.*, **6**, 39 (1980).
- [11] B.M. Lawrence. *Perfum. Flavor.*, **5**, 6 (1980).
- [12] G. Burkhardt, J. Reichling, R. Martin, H. Becker. *Pharm. Weekblad. Sci. Ed.*, **8**, 190 (1986).
- [13] K.H. Kubeczka, I. Bohn, V. Formacek. *Prog. Essent. Oil Res., Proc. Int. Symp. Essent. Oils*, **16**, 279 (1986).
- [14] P.M. Santos, A.C. Figueiredo, M.M. Oliveira, J.G. Barroso, L.G. Pedro, S.G. Deans, A.K.M. Younus, J.J.C. Scheffer. *Phytochem.*, **48**, 455 (1998).
- [15] P.M. Santos, A.C. Figueiredo, M.M. Oliveira, J.G. Barroso, L.G. Pedro, S.G. Deans, A.K.M. Younus, J.J.C. Scheffer. *Biotechn. Lett.*, **21**, 859 (1999).
- [16] H. Kollmannsberger, G. Fricke, H. Paulus, S. Nitz. *Adv. Food Sci.*, **22**, 47 (2000).
- [17] R. Omidbaigi, A. Hadjiakhoondi, M. Saharkhiz. *JEOBP*, **6**, 46 (2003).
- [18] M. Kurkuoglu, N. Sargin, K.H.C. Baser. *Chem. Nat. Compd*, **39**, 355 (2003).
- [19] V.M. Rodrigues, P.T.V. Rosa, M.O.M. Marques, A.J. Petenate, M.A.A. Meireles. *J. Agric. Food Chem.*, **51**, 1518 (2003).
- [20] *ESCOP monographs*, 2nd Edn, ESCOP, Thieme, Stuttgart, New York (2003).
- [21] N. Arslan, B. Gürbüz, E.O. Sarihan, A. Bayrak, A. Gümüşcü. *Turkish J. Agriculture and Forestry*, **28**, 173 (2004).
- [22] N. Tort, B. Honermeier. *Asian J. Chem.*, **17**, 2365 (2005).
- [23] A. Besharati-Seidani, A. Jabbari, Y. Yamini. *Anal. Chim. Acta*, **530**, 155 (2005).
- [24] N. Tabanca, B. Demirci, T. Ozek, N. Kirimer, K. Husnu Can Baser, E. Bedir, I.A. Khan, D.E. Wedge. *J. Chromatogr. A*, **1117**, 194 (2006).
- [25] N. Tabanca, B. Demirci, N. Kirimer, K. Husnu Can Baser, E. Bedir, I.A. Khan, D.E. Wedge. *J. Chromatogr. A*, **1097**, 192 (2005).
- [26] A. Velasco-Negueruela, M.J. Perez-Alonso, P.L. Perez de Paz, J. Pala-Paul, J. Sanz. *J. Chromatogr. A*, **1095**, 180 (2005).
- [27] M.G.L. Brandão, R.M.S. Alves, R.A. Moreira, P. Oliveira, M.T. Vieira, L.M. Moreira-Campos. *Rev. Bras. Plant. Med.*, **5**, 56 (2002).
- [28] D.A. Muravjova, I.A. Sam'lina, G.P. Yakovlev. *Farmakognozija*, Meditsina, Moskva (2002).
- [29] J.-G. Lee, Y.-J. Kwon, H.J. Jang, J.J. Kwag, O.C. Kim, Y.-H. Choi. *Han'guk Nonghwa Hakhoechi*, **40**, 144 (1997).