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Investigations on the Ratio of Methylchavicol and *Trans*-anethol Components in Essential Oil of Anis (*Pimpinella anisum* L.) from Different Regions of Turkey

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The objective of this study was to determine the ratio of methylchavicol and *trans*-anethol components in essential oil of *P. anisum* collected from four different regions of Turkey. The essential oil contents of the collected samples were determined with a Neo-Clevenger apparatus. The ratio of methylchavicol and *trans*-anethol components in essential oils was determined by gas chromatography. The essential oil content of the samples varied from 1.85 to 2.38%. Within the regions the ratio of methylchavicol ranged from 1.89 to 3.18% and the *trans*-anethol component varied from 95.57 to 97.24%.

Key Words: Anise plant, *Pimpinella anisum*, Essential oils, Methylchavicol, *Trans*-anethol.

INTRODUCTION

The anise plant, *Pimpinella anisum* L. (Apiaceae), has been grown in the Eastern Mediterranean countries for a very long time. It is commonly known as "enison, nanahan and raziyane-i rumi" in Turkey[1]. The plant has been used for different purposes since ancient times. Especially the essential oils produced from its fruits are utilized in pharmacy. Its important effects on human health are carminative, spazmolitic, diuretic, diaphoretic, bronchodilator, stomachic, expectorant, emmenagogue, lactogog and antiseptic when used in heavy doses[2–4]. The essential oils of *P. anisum* are also used in the beverage industry; for example, Turkish Raki, Greek Ouzo, French Pernod, Pastis and Anisette are some of the most famous beverages[5]. In addition, the residue of anise plant is used as fodder because of its high protein (18%) and fat (23%) contents[6]. Furthermore, Zargari[7] found that the anise plant regulates the functions of digestive system in animals and increases productivity of milk in cows.

P. anisum is widely cultivated in countries with hot or mild climate such as in Spain, Italy, Russia, India, Balkan countries, Turkey and Middle and South America[8]. In Turkey, it is mainly grown in the southern and western parts of

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Anatolia as in Burdur, Denizli, Antalya, Mugla, Bursa, Balikesir, Izmir, Çeşme, Afyon, Kütahya and Isparta. There are still doubts about the genetic origin of this plant species. Some researchers believe that it originates from the Mediterranean and Anatolian regions of Asia, but others believe it comes from the Far East countries. It is assumed that because of its wide and natural production in Turkey, Greece, Sicily and Egypt, the first opinion gains more support[9].

A large number of references are available in literature on the essential oils of *P. anisum*[7, 8, 10–13]. But such information was rare for the ecotypes of this plant species in Turkey[14–16]. Therefore, the objective of this study is to determine and compare the essential oil content and the two basic components of essential oils in *P. anisum* in different regions in Turkey.

EXPERIMENTAL

Similar aged fruits of *P. anisum* ecotypes were collected from four important growing areas of Antalya, Denizli, Çeşme and Burdur regions and brought into the laboratory for investigations. The ratio of essential oils in grains was volumetrically determined with a Neo-Clevenger apparatus and then calculated as percentage on dry material[15]. The analysis of the percentages of the essential oils was done two-folds as parallels. The ratio of methylchavicol and *trans*-anethol components in essential oils was determined by gas chromatography (2350 Model, Fractovap Series, Carlo Erba Company). The isothermal conditions of the apparatus were maintained as follows: length of the column: 3 m (glass column); material of the column: fixed phase: 3% OVI; support material: gas chrome Q; column temperature: 110°C; detector temperature: 250°C; injector temperature: 250°C; speed of translated gases: nitrogen: 25 mL/min, hydrogen: 30 mL/min, dry air: 300 mL/min; FID-detector, integrator: spectra physics; injecting sample: 0.5 μL (Hamilton); solvent used: chloroform.

RESULTS AND DISCUSSION

The essential oil ratio in four different ecotypes of *P. anisum* varied among the regions (Table-1). It was on an average 2.30% in Antalya, 2.23% in Denizli, 2.38% in Çeşme and 1.85% in Burdur. The results obtained by the earlier researchers on the essential oil ratios in Turkey and other countries of the world differ from one another and from the present results. But, some parts of the present results coincide in the lower and upper limits with the earlier ones. A Turkish researcher examined essential oil ratios in three naturally occurring *Pimpinella* species in Çeşme region of Turkey[17]. It was found that the essential oil ratio was 2.92% in *P. anisum*, 2.2% in *P. cretica* var. *cretica*, 0.85% in *P. tragium* ssp. *lithophila* and 0.40% in *P. tragium* ssp. *polyclada*. Another author reported[14] that the essential oil ratio in the fruits of *P. anisum* was 1.82% in Gölhisar and 2.9% in Urla regions of Eagean area. But other investigations observed much higher ratios of essential oils ranging from 8.3–8.7% in *P. anisetum*, 7.8–8.0% in *P. flabellifolia*, 10.3% in *P. cretica* var. *arabica* and 7.07–7.5% in *P. anisum*[4]. Scientists from Izmir (Turkey) determined the essential oil ratios in *P. anisum* L. in ecotypes of Antalya, Denizli, Fethiye and Çeşme[16]. They found the ratios

differed with a narrower variation of 2.10–2.38%. The same variation in the essential oil ratio has drawn attention to the reports of the foreign researchers, too. In other countries of the world, essential oil ratios determined in *P. anisum* also differed widely from 1.5 to 6.0%[8]. All these results show that the essential oil ratio in the fruits of *P. anisum* varies with different factors, like species, ecotypes, climate and characteristics of soil of the region.

TABLE-1
THE ESSENTIAL OIL RATIOS IN DIFFERENT ECOTYPES OF *PIMPINELLA ANISUM* FRUITS COLLECTED FROM DIFFERENT REGIONS OF TURKEY

Ecotypes	Average	Essential oil ratios (%)	
		1st repetition	2nd repetition
Antalya	2.29	2.31	2.30
Denizli	2.35	2.10	2.23
Çeşme	2.25	2.50	2.38
Burdur	1.75	1.95	1.85

The two basic components of methylchavicol and *trans*-anethol ratios determined from the essential oils of the fruits of *P. anisum* are presented in Table-2 and the original gas chromatography graphs are given in Figs. 1 and 2. Methylchavicol ratios varied among the four regions. It was 1.89, 2.09, 3.18 and 1.92% for Antalya, Denizli, Çeşme and Burdur, respectively. The results of these studies are more or less similar to the findings of Fehr[12] with 1.3–2.5% but it is highly different from the results of El-Wakeil *et al.*[11] with 4.95% ratio.

TABLE-2
METHYLCHAVICOL AND *TRANS*-ANETHOL RATIOS IN DIFFERENT ECOTYPES OF *PIMPINELLA ANISUM* FRUITS COLLECTED FROM DIFFERENT REGIONS OF TURKEY

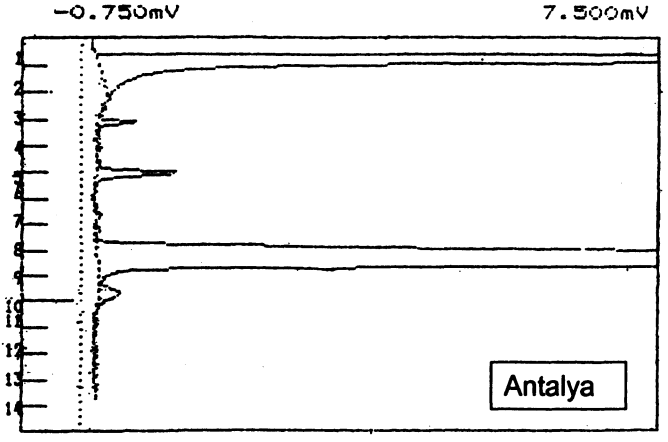
Ecotypes	Methylchavicol (%)	*Trans*-anethol (%)
Antalya	1.89	96.57
Denizli	2.09	97.24
Çeşme	3.18	95.57
Burdur	1.92	96.55

The *trans*-anethol ratios also differed among the four regions. It varied from 96.57, 97.24, 95.57 and 96.55% for Antalya, Denizli, Çeşme and Burder regions, respectively. The results of Kaya[14] with variations from 95.84 to 97.18% in *trans*-anethol ratios for *P. anisum* ecotypes are almost similar to the present findings. But on the contrary the results of Tort[17] with a very wide variation from 3.52 to 95.54% ratios in wild Anise species showed great differences and only the upper limit of the ratios coincides with the results of this study.

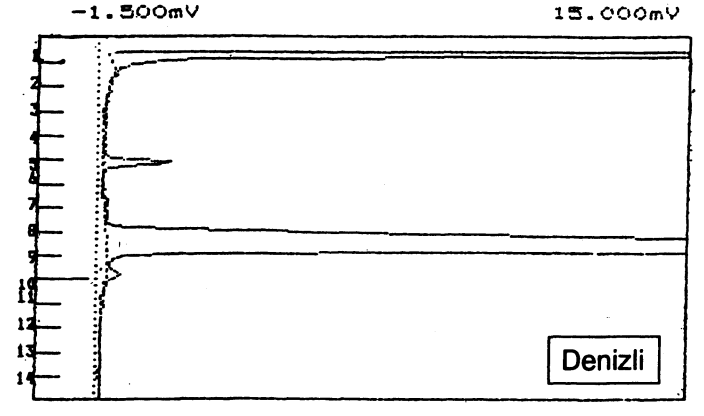
In literature, great variations can be found for *trans*-anethol ratios even for the same anise plant species, for example, the ratio in *P. anisum* was 95.5%[17] between 80 and 90%[4] and between 73.5 and 85.2%[18]. Although the earlier workers have reported wide variations in *trans*-anethol ratios for anise plant species, yet these variations are not so high as in the present studies.

In some other countries of the world also, the anethol ratios show variations. The *trans*-anethol ratio was highest in Hungary and Spain with 97.4% and 95.2%[10], in Bulgaria with 87.3%[19] and in Egypt with 86.2%[11]. Some other authors reported an anethol ratio from 80 to 90%[8, 17] and from 89.7 to 94.1%[12, 19].

Besides these two components of methylchavicol and *trans*-anethol, there are some other components determined by various authors. Tort[17] has listed borneol, cineol, linalool, α-pinene, β-pinene and terpinene as some of the other components of essential oils of four species of anise plant. El-Wakeil *et al.*[11] worked on the

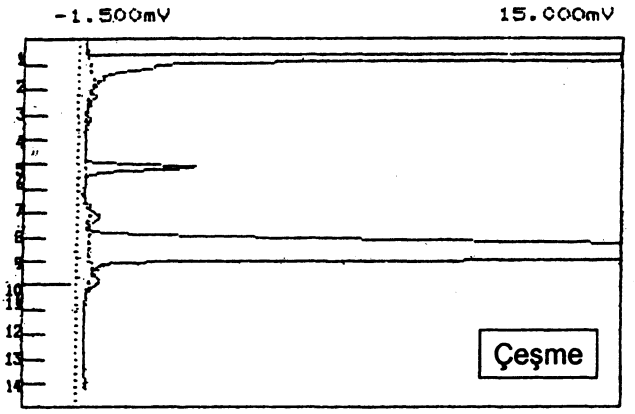


RETENTION	AREA	AREA %	COMPONENTS
5.033	16.14	1.89	Methylchavicol
8.500	823.16	96.57	Trans-anethol

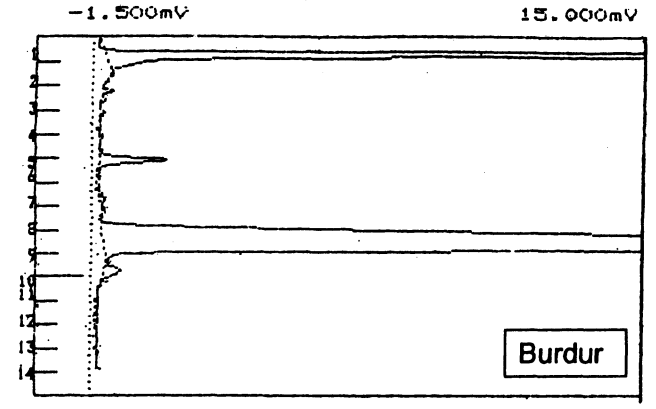


RETENTION	AREA	AREA %	COMPONENTS
5.083	26.67	2.09	Methylchavicol
8.733	1242.70	97.24	Trans-anethol

Fig. 1. Chromatography graphs of essential oil from Pimpinella anisum, Ecotypes Antalya and Denizli



RETENTION	AREA	AREA %	COMPONENTS
5.083	50.76	3.18	Methylchavicol
8.833	1524.72	95.57	Trans-anethol



RETENTION	AREA	AREA %	COMPONENTS
5.050	29.40	1.92	Methylchavicol
8.766	1482.10	96.55	Trans-anethol

Fig. 2. Chromatography graphs of essential oil from Pimpinella anisum, Ecotypes Cesme and Burdur

Egyptian origin anise plant and isolated quantitatively *p*-cymene, caryophyllene, linalool, linalyl acetate, anisketone, anis alcohol, anisic acid and eugenol from the various components of essential oils.

According to the researchers, many factors are effective on the components of the essential oil of anise plant. Kubeczka and Bohn[19] observed that essential oils especially the contents of anethol in different organs and tissues of the plant are different, for example, the anethol ratio was up to 94.1% in fruits while it was 29.4% at above ground tissues and only 3.4% in the roots. Tort[17] stated that the essential oil ratio in *Pimpinella cretica* species is higher than in *P. anisum* and *P. tragium*. Bayram[16] found the effects of some factors on the essential oil ratios and come to

the conclusion that fertilizer dose and types of sowing do not change the essential oil ratio.

The two basic components of methylchavicol and *trans*-anethol of essential oils of *P. anisum* vary not only among regions but also it varies for the same plant species in the same region. Many other factors might be responsible for the variations in essential oil components, which have not been listed in the literature and should be investigated in future.

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