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RESEARCH ARTICLE

Chemical diversity of essential oils within the *Origanum dubium* Boiss. population

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Abstract

Origanum dubium Boiss. is one of the economically important wild oregano species and it is intensely collected from its natural habitat in Antalya, Turkey. Carvacrol chemotype of *Origanum dubium* is used mainly for essential oil production due to its high essential oil content. In this study, chemical diversity of *Origanum dubium* was investigated in order to develop new cultivars with improved essential oil yield and carvacrol content using clone selection method under cultivated condition. Essential oils obtained by hydrodistillation of the aerial parts of *Origanum dubium* were analysed by GC-MS and 24 major components were identified. Among the selected genotypes, carvacrol was the major component and followed by *p*-cymene, γ–terpinene, myrcene and α-thujene, respectively. The essential oil yields were varied between 5.0% to 14.0%; carvacrol rates were varied between 73.76% to 88.21%; and *p*-cymene rates were varied between 3.16% to 9.10%.

Keywords: *Origanum dubium*, essential oil, carvacrol, diversity

Introduction

Origanum dubium Boiss. is one of the economically important wild oregano species in Turkey. The most important characteristic of this oregano is high essential oil content (6-8%). It is intensely wild crafted from the upland of Alanya and Gazipasa towns of Antalya province and used for essential oil production (Turgut et al., 2017). However, there are various genotypes and chemotypes within the wild populations such as high carvacrol and high linalool types (Turgut et al., 2017). Also, natural populations have been decreased year after year. Therefore, cultivation of *Origanum dubium* seems to be the most convenient way for conservation of wild populations and production of stable drugs.

Origanum dubium Boiss. is grown in the wild flora of Turkey, Greece and Cyprus. Recently, A new basis regarding the taxonomic uncertainties concerning section “Majorana” was reported (Lukas et al., 2010; Lukas et al., 2013). It was assessed and discussed that the taxonomic status of *O. onites*, *O. syriacum*, *O. dubium* and *O. majorana* and discuss evolutionary relationships in section Majorana by considering molecular, morphological and phytochemical evidence. Accordingly, it was concluded that, "cymyl" chemotype of *Origanum majarona* L. is classified as *Origanum dubium* Boiss. It is known that the carvacrol-rich species in the flora of Turkey which was published as *O. majorana* is actually *O. dubium* (Lukas et al., 2013).

The leaves and flowers are used against gastrointestinal problems, while its essential oil is used as an antirheumatic (Arnold et al., 1993). In addition, essential oil of *O. dubium* is evaluated as industrial products in many areas (Vera & Ming, 1999) due to its antimicrobial and potential antioxidant activity (Karioti et al., 2006). The biological activities of essential oil of *Origanum dubium* Boiss. such as fungicidal (Ahmad et al., 2011; Dambolena et al., 2011), insecticidal (Tang et al., 2011), antimicrobial (Nostro & Papalia, 2012),

anticarcinogenic (Koparal & Zeytinoğlu, 2003) and antioxidant properties (Mezzoug et al., 2007) associated with carvacrol content. Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a natural biological compound and it is a monoterpene phenol.

Recently, utilization of oregano oils has been increased significantly in various sectors (food, health, agriculture, cosmetics etc.). Thus, the yield of essential oil and carvacrol in oregano is very important due to its biological activity. *Origanum dubium* is used mainly for essential oil production not for culinary herb or herbal tea production because of high essential oil rate. Therefore, development of new genotypes with much higher essential oil yield and carvacrol content will be very valuable for the industry. The aim of this present study was to determine chemical diversity of *Origanum dubium* Boiss. related to agricultural conditions, in order to develop new cultivars with improved essential oil yield and carvacrol content.

Materials and Methods

Plant material

In the preliminary studies, high essential oil and carvacrol type *Origanum dubium* Boiss. populations were identified and their seeds were collected from the natural flora of Gazipasa towns of Antalya province in Turkey (1372 m above sea level and N36 26.749 E32 28.266). Specimens were identified by R. S. Göktürk in the Department of Biology, Akdeniz University. Seeds were germinated in the greenhouse and then 2200 healthy seedlings were transferred to the experimental plot for individual plant selection. One hundred plants (genotypes) were selected according to their agronomic features and essential oil yields. After that, 10 stem cuttings from each plant (genotype) were rooted in the greenhouse condition and then they were planted in a row, thus in total 100 rows with 1000 plants were established in open field. Each row with 10 clones was called as a different genotype. The study was conducted in Antalya located in Mediterranean Region of Turkey (33 m above sea level and 36 ° 53’ N; 30° 38’ E). This location was characterized by a Mediterranean climate with 1068 mm total rain fall, 18.7 °C mean air temperature, 13.6° C minimum air temperature and 24.2 maximum air temperature. Terra-rossa type soil characteristics of the experimental field were clay loam, very high in lime, low in salt, and light alkaline (pH 7.7). The layer of 0-30 cm soil had low concentrations of organic material and sufficient amount of nitrogen. Available phosphorus content of the soil was low and useful potassium content was high.

Essential oil isolation

Plant rows which consist of 10 plants (clones) were harvested separately and then aerial parts were dried at room temperature. For the study, 100 g of samples from each plant row were subjected to water distillation for 3 h using a Clevenger type apparatus. As a result, percentage of essential was measured by the volumetric method (v/w) for each sample.

Analysis of the essential oils

Samples were diluted 1:50 with hexane for analyses. GC-MS analyses were performed on a gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C) GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250 °C). Temperature was programmed from 60°C (10 min.) to 250 °C (10.5 min.) and analysis was carried out in a total time of 60 minutes. Helium was used as carrier gas at the flow rate of 0.8 ml/min. The capillary column used was HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm. Samples were injected 1 µL with 50:1 split rate. The identification of the components of *Origanum dubium* Boiss essential oil was confirmed by comparison of their relative retention times and mass spectra

with OIL ADAMS, NIST and Wiley libraries. Retention indices of all the components were determined by the Kovats method.

Results and Discussion

In this study, yield and composition of the essential oil from aerial parts of 100 selected *Origanum dubium* Boiss. genotypes were determined. Among 100 genotypes, only eight samples which represented chemically different groups were chosen for presenting chemical diversity within the population. Chemical constituents of the eight samples with retention index are shown in Table 1. In total, 24 different components were identified representing 98.46% to 99.68% of the essential oil by GC–MS analysis. Quantitatively, carvacrol was the major component and followed by *p*-cymene, γ-terpinene, myrcene and α-thujene. All compounds except carvacrol were found in much lower amounts (Table 1). They were rich in the active monoterpene phenols such as carvacrol and monoterpene hydrocarbon precursors such as *p*-cymene and γ-terpinene. "Cymyl" pathway accumulates of γ-terpinene, *p*-cymene, carvacrol, thymol, and this compounds are characteristic for a number of *Origanum* species, e.g., *O. dubium* Boiss., *Origanum onites* L., and *Origanum syriacum* L. (Skoula & Harborne, 2002; Lukas et al., 2013).

According to the results, essential oil yields were varied between 5% to 14% (Table 1). Actually, 24 genotypes out of one hundred were produced more than 10.0% essential oil yield; 62 genotypes produced at least 8.0% essential oil yield (data is not given). These essential oil yields were found to be much higher than earlier studies which were reported as 7.6% (Sarer et al., 1982) and 6.5 - 7.7% (Baser et al., 1993). Among the selected genotypes only one genotype (H) produced extremely high (14%) essential oil yield with high carvacrol (84.65%) content (Table 1). The main constituent was carvacrol in all of the genotypes and carvacrol rates were varied between 73.76% to 88.21% with average rate of 83.29% (Table 1). These results showed presence of significant variations in carvacrol content of *O. dubium*. The highest carvacrol rates were obtained from genotype F (88.21%) and followed by genotype genotype C (86.87%), genotype D (86.16%) and genotype B (85.88%). Carvacrol contents of the genotypes were found to be higher than previous studies on O. *majarona* from Turkey 78.27 - 79.46% (Baser et al., 1993) and *O. dubium* from Cyprus 69.5 - 71.3% (Karioti et al., 2006). Second major constituent was appeared to be *p*-cymene in all samples and it varied between 3.16% to 9.10% (Table 1).

According to the results, the highest *p*-cymene (9.10%) rate was obtained from genotype G which had also the lowest carvacrol rate (73.76%). On the other hand, samples (genotypes C and F) which produced lower rates of *p*-cymene were found to have higher carvacrol rates (Table 1). These results showed that carvacrol and *p*-cymene ratios were inversely correlated. Chemotypes which with specific enzymatic equipment are genetically codified and direct their biosynthesis to the preferential formation of a definite compound (De Martino, 2009). In the case of phenolic compounds, the metabolic pathway is through the auto oxidative conversion of γ-terpinene to *p*-cymene followed by hydroxylation of *p*-cymene to thymol or carvacrol (Poulose & Croteau, 1978).

In all samples, γ-terpinene was found to be third abundant component which varied from 1.14% to 5.17% (Table 1). Similar to the state of *p*-cymene / carvacrol, genotype G which produced the lowest carvacrol rate (73.76%) and the highest *p*-cymene rate (9.10%), also produced the highest γ-terpinene rate (5.17%). On the contrary, genotype F which gave the highest carvacrol rate (88.21%), gave the lowest γ-terpinene rate (1.14%). Same as *p*-cymene, inverse correlation between carvacrol and *γ*-terpinene ratios could be explained by "Cymyl" pathway of *Origanum* species. Aligiannis et al. (2011) reported that rate of γ-terpinene was 4.66% in *Origanum scabrum*. Figuérédo et al. (2006) studied the composition of the oils of

six species of carvacrol-rich Mediterranean oregano and they found that the amounts of γ-terpinene (1-15%) and *p*-cymene (1-9%) were more abundant in *O. compactum*, *O. dictamnus*, *O. dubium*, *O. minutiflorum*, *O. onites* and *O. vulgaris* ssp. *hirtum*.

Apart from those three constituents, genotype A had borneol (1.32%) and trans-sabinene hydrate (1.28%); genotype B had α-thujene (1.09%) and *trans*-sabinene hydrate (1.06%); genotype C had *trans*-sabinene hydrate (1.11%) and myrcene (1.08%); genotype D had myrcene (1.11%) and α-thujene (1.09%); genotype E had myrcene (1.22%) and linalool (1.10%); genotype F had α-thujene (0.95%) and linalool (0.95); genotype G had *trans*-sabinene hydrate (1.60%) and α-terpinene (1.34%); genotype H had myrcene (1.22%) and α-thujene (1.22%).

Carvacrol, linalool, linalool-carvacrol chemotypes as well as thymol chemotypes were found in *O. dubium* (Figuérédo et al., 2006). Thymol, linalool and carvacrol chemotypes were reported in Turkish *O. dubium* populations, while Cyprus ones revealed only carvacrol chemotype (Figuérédo et al., 2006). Essential oil compositions are appeared to be different from plant to plant, population to population and one locality to other locality, because chemical profile is highly influenced by genetic and environmental factors.

Table 1. Essential oil composition of selected *Origanum dubium* genotypes.

Peak No	RI*	Compounds	Genotype A (%)	Genotype B (%)	Genotype C (%)	Genotype D (%)
Essential oil yield %			**6**	**8.5**	**11**	**5**
1	1019	methyl-2-methylbutyrate	0.108	0.072	0.07	0.08
2	1030	α-pinene	0.612	0.466	0.41	0.49
3	1033	α-thujene	1.16	1.097	0.94	1.09
4	1080	camphene	0.377	0.09	0.09	0.09
5	1123	β-pinene	0.182	0.14	0.13	0.13
6	1168	myrcene	1.133	1.058	1.08	1.11
7	1179	α-phellandrene	0.187	0.145	0.15	0.16
8	1190	α-terpinene	1.045	0.558	0.52	0.63
9	1213	limonene	0.24	0.127	0.12	0.13
10	1223	1.8-cineole	0.392	0.311	0.35	0.24
11	1257	γ-terpinene	4.116	1.687	1.48	1.40
12	1283	*p*-cymene	5.805	3.925	3.16	4.21
13	1447	1-octen-3-ol	0.154	0.175	0.13	0.11
14	1470	*trans*-sabinene hydrate	1.28	1.068	1.11	0.94
15	1549	linalool	0.139	0.176	0.13	0.16
16	1560	*cis*-sabinene hydrate	0.383	0.29	0.28	0.30
17	1616	terpinen-4-ol	0.561	0.561	0.54	0.57
18	1625	β-caryophyllene	0.512	0.087	0.17	0.26
19	1712	α-terpineol	0.261	0.208	0.28	0.22
20	1717	borneol	1.323	0.317	0.40	0.26
21	1749	carvone	0.171	0.249	0.20	0.14
22	2143	spathulenol	0.095	0.066	0.09	-
23	2187	thymol	0.633	0.693	0.68	0.69
24	2220	carvacrol	78.037	85.88	86.87	86.16
		Total identified (%)	**98.90**	**99.44**	**99.38**	**99.56**

*RI: Retention Index, - : not detected

Table 1 (continued)

Peak No	RI*	Compounds	Genotype E (%)	Genotype F (%)	Genotype G (%)	Genotype H (%)
Essential oil yield %			**6.5**	**8**	**8.5**	**14**
1	1019	methyl-2-methylbutyrate	0.09	0.09	0.12	0.13
2	1030	α-pinene	0.56	0.43	0.59	0.56
3	1033	α-thujene	1.08	0.95	1.31	1.22
4	1080	camphene	0.17	0.09	0.20	0.16
5	1123	β-pinene	0.17	0.12	0.20	0.16
6	1168	myrcene	1.22	0.90	1.19	1.22
7	1179	α-phellandrene	0.18	0.12	0.17	0.17
8	1190	α-terpinene	0.80	0.46	1.34	0.66
9	1213	limonene	0.17	0.10	0.27	0.15
10	1223	1.8-cineole	0.42	0.18	0.65	0.26
11	1257	γ-terpinene	2.55	1.14	5.17	1.74
12	1283	*p*-cymene	4.22	3.39	9.10	4.77
13	1447	1-octen-3-ol	0.06	0.07	0.17	0.08
14	1470	*trans*-sabinene hydrate	0.19	0.10	1.60	1.03
15	1549	linalool	1.10	0.95	0.10	0.08
16	1560	*cis*-sabinene hydrate	0.11	0.15	0.40	0.30
17	1616	terpinen-4-ol	0.32	0.26	0.58	0.55
18	1625	β-caryophyllene	0.63	0.11	0.19	0.07
19	1712	α-terpineol	0.24	0.27	0.47	0.19
20	1717	borneol	0.60	0.24	0.60	0.51
21	1749	carvone	0.21	0.13	0.18	0.26
22	2143	spathulenol	-	-	0.18	0.11
23	2187	thymol	0.67	-	0.60	0.67
24	2220	carvacrol	82.76	88.21	73.76	84.65
		Total identified (%)	**98.53**	**98.46**	**99.15**	**99.68**

*RI: Retention Index, - : not detected

Conclusion

Considerable variations in essential oil yield and constituents were found within the population of O. dubium which were collected from the wild flora and then grown in the field. Essential oil yields were varied between 5% to 14%. As expected, carvacrol was the major constituent and its rates were varied from 73.76% to 88.21. These variations proved that wild O. *dubium* populations are genetically and chemically heterogeneous. Selection and cultivation of oregano genotypes with high essential oil yield and carvacrol content are believed to be very important for essential oil producers and other related sectors.

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