

Growth Stage and Drying Methods Affect Essential Oil Content and Composition of Pickling Herb (*Echinophora tenuifolia* subsp. *sibthorpiana* Tutin)

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Abstract: The present research was conducted during 2012 in order to determine the essential oil content and composition of *Echinophora tenuifolia* subsp. *sibthorpiana* Tutin. Plants were collected during rosette, vegetative growth, full flowering and fruit-ripening stages. Oil was extracted using Clavenger hydro-distillation apparatus from either fresh, shade dried or sun dried samples. Oil composition was determined with a GC/MS. Oil content of samples showed significant variation during the vegetative stages of development. Oil contents of fresh samples were found to be 0.76% at seedling stage whereas oil content has risen to 1.06% at seed set. The shade-dried samples had higher oil contents than the fresh and sun dried samples. The oil composition of pickling herb changed with drying method and growth stage. Throughout the growth stage of the plant, the oil was composed of 21 components and the main components were found to be α -phellandrene (47.43 - 66.39%) and methyl eugenol (21.29 - 38.72%). While methyl eugenol content decreased during vegetation period for both fresh and dried samples, α -phellandrene level increased. Attention should be given to the collection time and drying method of pickling herb for different uses since vegetative stage and drying method influence oil content and composition.

Gelişme Dönemi ve Kurutma Şeklinin Çörtük Otu (*Echinophora tenuifolia* subsp. *sibthorpiana* Tutin)'nin Uçucu Yağ Oranı ve Bileşenleri Üzerine Etkilerinin Belirlenmesi

Anahtar Kelimeler

Echinophora tenuifolia
subsp. *sibthorpiana*
Gelişme dönemi
Kurutma şekli
Uçucu yağ oranı ve
bileşenleri

Özet: Araştırma, Isparta florasında yaygın olarak yetişen çörtük otu (*Echinophora tenuifolia* subsp. *sibthorpiana* Tutin) bitkisinin gelişme dönemi boyunca uçucu yağ oran ve bileşenlerinin belirlenmesi amacıyla 2012 yılında yürütülmüştür. Bitkiler fide, sapa kalkma, tam çiçeklenme ve tohum bağlama dönemlerinde toplanmıştır. Uçucu yağ analizleri hem taze olarak hem de gölge ve güneşte kurutulduktan sonra yapılmıştır. Örneklerdeki uçucu yağ miktarları clavenger tipi hidro-distilasyon cihazında, uçucu yağ bileşenleri ise GC/MS cihazında belirlenmiştir. Bitkilerin uçucu yağ oranlarının gelişme dönemlerine göre önemli varyasyonlar gösterdiği, taze örneklerde fide döneminde % 0.76 olan uçucu yağ oranının tohum bağlama döneminde % 1.06'ya yükseldiği belirlenmiştir. Gölgede kurutulan örneklerin uçucu yağ oranları taze ve güneşte kurutulan örneklerle göre daha yüksek bulunmuştur. Uçucu yağ bileşenleri bitki gelişme dönemine ve kurutma yöntemine bağlı olarak önemli değişimler göstermiştir. Bitkinin tüm gelişme dönemlerinde de uçucu yağın yaklaşık 20 farklı bileşenden oluştuğu ve ana bileşenlerin α -phellandrene (% 47.43 - 66.39) ve methyl eugenol (% 21.29 - 38.72) olduğu belirlenmiştir. Hem taze hem de kurutulan örneklerde gelişme dönemi ilerledikçe methl eugenol azalırken, α -phellandrene artış göstermiştir. Çalışmada, çörtük otu bitkisinde gelişme dönemi ve kurutma yönteminin uçucu yağ oranı ve bileşenleri üzerine önemli etkiler gösterdiği ve bitkinin kullanım amacına bağlı olarak hasat zamanı ve kurutma yönteminin dikkate alınması gerektiği sonucuna varılmıştır.

1. Introduction

The genus *Echinophora* (Apiaceae) comprises about 10 species, distributed from the Mediterranean region to Afghanistan [1]. The Mediterranean and Middle East regions seem to be the only areas where this genus is established [2]. There are three taxa of the genus found in Europe (*E. spinosa*, *E. tenuifolia* ssp. *sibthorpiana* and *E. tenuifolia* ssp. *tenuifolia*) [3]. The flora of Turkey contains six species, three of which are endemic. Fresh or dried *E. tenuifolia* is used in the treatment of wounds, gastric ulcers and digestive activities in folk medicine due to its antifungal, carminative and digestive properties [4]. It is also added to foods, such as; soup, meat, pickles, dairy products, and meatballs, for enhancing their sensory properties [5, 6].

Oil composition depends upon both biotic (genetic, ontogeny, morphogenesis) and abiotic (climate, soil, temperature etc.) factors affecting plant growth. Morphogenetic variability is the variation of oil compositions according to different parts of the plant, such as; flower, leaf, root etc. [7]. The post-harvest process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active ingredient in the product sold. Aromatic plants are often dried before extraction to reduce the moisture content of the product to a level that prevents deterioration of the product and allows storage in a stable condition. Proper drying of medicinal plants is fundamental to the achievement of a high quality product [8]. In addition, the main purpose of drying is to extend product shelf life, minimize packaging requirements and reduce shipping weights [9]. It has been showed that drying method had a significant effect on oil content and composition of aromatic plants [10-14].

Some studies on the essential oil composition of *Echinophora* species have been carried out [2, 6, 14-19]. Most of the studies dealt with components identified from different locations and growth stages, however; changes of essential oil content and composition at different plant growth stages with response to drying methods has not been investigated. The objective of the current study was to investigate the effect of growth stages and drying methods on essential oil content and chemical components of *E. tenuifolia* subsp. *sibthorpiana* Tutin from the Lakes Region of Turkey (Isparta).

2. Material and Method

2.1. Plant material

The experiment was carried out at the Field Crops Department of Suleyman Demirel University, Isparta, Turkey in the summer of 2012 (elevation 1030 m, average annual rainfall: 500 mm, and mean annual temperature: 18°C). Plant samples were collected at

the different plant growth stages (rosette, vegetative growth (before flowering), full flowering and seed maturing stage) from the Research Farms of Suleyman Demirel University. The investigated drying methods were shade and sun drying. Five plants were used for each developmental period. The first group of samples was shade-dried at room temperature for 5 days till they reached to a constant weight. The second group was dried under the sun for 3 days until reaching a constant weight. Plant material was distributed as a thin layer to accelerate drying process. The maximum daily temperature during drying process ranged from 25 to 30°C.

2.2. Essential oil extraction

The fresh and dried plant materials (250 g) were powdered and used for extraction by using a hydro-distillation technique during 3 hours in an all-glass Clevenger type apparatus that separates water from oil. The essential oils were separated from the aqueous layer and essential oil yield was calculated. The extracted oil was stored in a dark glass tube and kept under refrigeration at 4°C until further analysis. Extraction was carried out in triplicates.

2.3. Identification of components

GC-MS (Gas Chromatography-Mass Spectrometry) analysis of the oil samples was performed on a QP5050 GC-MS equipped with a Quadrapole detector. GC/MS analysis was conducted under the following conditions: capillary column, CP-Wax 52 CB (50 m x 0.32 mm; film thickness = 0.25 µm); oven temperature program (60 °C increased to 220 °C at a rate of 2 °C /min and then kept at 220 °C for 10 min); total run time 60 min; injector temperature, 240 °C; detector temperatures, 250 °C; carrier gas, helium at a flow rate of 20 ml/min. Relative percentage amounts of the essential oil components were evaluated from the total peak area (TIC) by apparatus software. Identification of components in the volatile oil was based on the comparison of their mass spectra and retention time with literature data and by computer matching with NIST and WILEY libraries [20].

Data was subjected to the analysis of variance (ANOVA) procedure with SAS statistical program [21]. Means were separated using LSD test at the 0.05 significance level.

3. Results

The analysis of variance showed that the plant growth stages and drying methods had a significant effect ($p < 0.01$) on the essential oil content of *E. tenuifolia* subsp. *sibthorpiana*. Essential oil contents obtained from different organs with different drying methods are given in Table 1. Essential oil content increased progressively during different growth and

developmental stages; from rosette stage to fruit-ripening stage. Essential oil contents of freshly analyzed plants at rosette, vegetative growth, full flowering and fruit-ripening stages were 0.76%, 0.87%, 0.97% and 1.06%, respectively. Essential oil contents of shade-dried (2.2 fold) and sun-dried (1.5 fold) samples were higher than the freshly analyzed samples (Table 1). The highest amount of essential oil was obtained from shade-dried plants at fruit-ripening stage (2.09%) and full flowering stage (2.0%) while the lowest value (0.76%) was obtained from fresh samples at rosette stages (Table 1).

The increase in essential oil content during the growth period is believed to be due to both the increased synthesis of the essential oils during the flowering period and decrease of the plant water content with maturation. Braga et al. [22] found that the essential oil content was increased by the removal of moisture from leaves. Observed increase of the essential oil is due to decrease of the moisture content of the dried samples. Aromatic and medicinal plants are often dried before extraction to reduce moisture content. During this process, many compounds which are dragged to the leaf surface by the evaporating water are lost [23]. The proposed mechanism explains the changes of essential oil content with drying procedure. The essential oil content of the samples dried under the light was 30% lower than the samples dried under shade conditions. The loss of volatile compounds may be due to the effect of exposure to direct sunlight and high temperature. The method of drying has a significant effect on the quality and quantity of the essential oils [24]. Generally, drying the plant material before distillation resulted in both increased and reduced essential oil yield depending on drying duration and temperature [9]. Shade-dried plants had higher essential oil than fresh and sun-dried samples were reported by Hamrouni-Sellami et al. [9]. Asekun et al. [10] stated that drying aromatic plants at air ambient temperature is the most efficient method in terms of essential oil yield. Similar to our results, the reduction of volatile oil content of plant by the impact of high temperature drying process has also been reported [13, 25-27].

It was reported that the essential oil content of *E. tenuifolia* subsp. *sibthorpiana* ranged between 0.2-2.4% depending on the location and plant growth stages [6, 17, 19, 28, 29]. Our results are similar to those reported by Ozcan and Akgul [19], who reported that the essential oil content of plants harvested in April, May and June was 0.9%, 1.3% and 1.1% respectively. Chalchat et al. [28] also reported that the oil content on a dry weight basis varied from 0.76% to 2.4% in plants that growing different locations. Telci and Hisil [9], reported that the yield of essential oils were found to be 0.20-0.23% in plants collected during rosette period and 0.40% in plants collected at pre-flowering period. However, Ghani et

al. [30] reported that the essential oil content of *E. platyloba* were 0.7, 0.5, and 0.2% in rosette, floral budding and full flowering stages, respectively.

Table 1. Essential oil content of *E. tenuifolia* subsp. *sibthorpiana* at different growth stages and in response to different drying methods

Growth Stages	Fresh herb	Shade drying	Sun drying
Rosette	0.76j	1.70bc	1.10fg
Vegetative growth	0.87ij	1.79b	1.28ef
Full flowering	0.97hi	2.00a	1.43de
Fruit-ripening	1.06gh	2.09a	1.59dc

Mean values with different superscript letters in the same column differ significantly according to LSD test ($P \leq 0.05$)

The chemical composition of *E. tenuifolia* subsp. *sibthorpiana* essential oils are summarized in Table 2. Approximately 20 compounds were identified in all samples, representing more than 98.2% of the total oil. The main components of essential oils were found to be α -phellandrene (47.43-66.39%) and methyl eugenol (21.29-38.72%). Other major constituents were myrcene (0.96-2.73%), limonene (0.82-1.40%), 1,8 cineole (0.81-3.21%), terpinolene (0.46-2.96%), α -terpinene (0.95-2.18%) and p-cymene (0.73-3.28%) (Table 2).

The quantities of the major compounds of essential oil have changed during various growth and developmental stages and in response to different drying methods. Myrcene, 1,8 cineole and α -terpinene percentages at the rosette stage and geraniol at the vegetative growth period were higher than the other growth stages in fresh samples. α -terpinene and limonene percentages increased after the full flowering stage (Table 2). One of the main components, methyl eugenol content decreased gradually from rosette to fruit-ripening stage, while the other main component, α -phellandrene increased gradually during growth and developmental stages (Table 2). At rosette stage α -phellandrene epoxide and at fruit ripening stage sabinene, α -terpinene and borneol were not detected (Table 2).

While α -terpinene and p-cymene contents were higher at fresh samples of all growth stages, limonene, 1,8 cineole and α -phellandrene contents increased with drying, especially with the sun drying method. However, drying process caused decreases in the α -terpinene, α -pinene and methyl eugenol contents. Caryophyllene oxide was detected only in fresh samples. On the other hand, α -thujene, which was detected in the oil at fresh and shade-dried samples, was not identified at the sun-dried samples. The results of essential oil analysis showed that the amounts of α -thujene and mentha-1,5-dien-8-ol components was relatively stable in all growth and developmental stages and drying methods. On the other hand, some components showed a fluctuation based on the different growth stages and drying methods (Table 2).

Methyl eugenol and α -phellandrene were the major constituents at different growth and developmental stages (Table 2). Methyl eugenol content of the essential oils decreased during the growth period depending on the drying methods. The decrease in methyl eugenol from rosette to fruit-ripening stages at shade (37.68-24.12%, respectively) and sun drying

(36.87-22.14%, respectively) samples were higher than fresh samples (38.72-33.8%, respectively). The amount of α -phellandrene increased as plants developed and α -phellandrene levels were higher at dried samples as compared to fresh samples.

Table 2. The changes in essential oil composition of *E. tenuifolia* subsp. *sibthorpiana* at different growth stages and in response to drying methods.

Essential Oil Components	RI	Rosette			Vegetative growth			Full flowering			Fruit-ripening		
		Fresh	Shade	Sun	Fresh	Shade	Sun	Fresh	Shade	Sun	Fresh	Shade	Sun
α -Thujene	929	0.18	0.14	t	1.16	0.22	t	0.41	0.16	t	0.28	0.21	t
α -Pinene	938	0.57	0.45	0.18	0.41	0.22	0.29	0.26	0.21	t	0.27	0.11	t
Sabinene	974	0.43	0.21	0.16	0.23	t	t	0.14	0.11	t	-	-	-
Myrcene	990	2.73	2.01	0.99	0.96	1.46	1.81	1.75	1.10	1.71	1.69	2.02	0.56
α -Phellandrene	1003	47.43	51.20	52.88	48.10	51.85	59.09	52.25	58.8	66.39	53.44	63.91	65.06
α -Terpinene	1015	0.17	0.11	0.13	0.19	0.15	0.24	0.12	t	t	-	-	-
<i>p</i> -Cymene	1024	1.18	0.67	1.09	3.28	1.52	1.71	1.41	1.28	1.01	1.36	0.78	0.73
Limonene	1030	0.93	1.05	1.40	0.82	1.06	1.14	1.07	1.17	1.35	1.01	1.3	1.4
1,8 Cineole	1034	1.30	1.49	1.88	0.81	0.93	1.02	0.90	1.53	2.58	0.88	1.86	3.21
(<i>Z</i>)- β -ocimen	1042	0.12	0.16	t	0.14	t	t	0.15	0.11	0.14	0.17	t	t
γ -Terpinene	1060	1.47	1.31	1.42	1.27	1.08	0.95	2.18	1.97	1.78	1.92	1.67	1.41
Terpinolene	1086	2.96	1.74	1.13	1.88	1.7	0.46	0.73	0.67	0.97	2.27	1.33	3.02
Linalool	1101	0.11	t	t	0.15	t	0.11	0.14	0.11	0.12	0.11	t	t
Isophorone	1122	t	-	-	t	t	t	0.17	0.21	0.36	0.33	0.25	0.21
Borneol	1157	0.14	0.11	0.18	0.21	0.15	t	0.27	0.12	t	-	-	-
Mentha-1,5-dien-8-ol	1174	0.21	0.24	0.18	0.35	0.47	0.41	0.22	0.28	0.32	0.41	0.25	0.29
α -Phellandrene epoxide	1197	-	-	-	0.23	0.18	0.27	0.21	0.16	0.13	0.12	t	t
Verbenane	1206	t	t	t	0.16	0.22	0.14	0.19	0.22	0.16	0.32	0.38	0.25
Geraniol	1249	0.39	0.23	0.16	0.48	0.42	0.55	0.23	0.21	0.25	0.17	0.13	0.11
Methyl eugenol	1395	38.72	37.68	36.87	38.11	36.65	30.11	36.12	30.41	21.29	33.89	24.12	22.14
Caryophyllene oxide	1582	0.16	t	t	0.18	t	t	0.23	t	t	0.14	t	t
TOTAL		98.97	98.64	98.65	98.83	98.28	98.19	98.86	98.61	98.30	98.50	98.32	98.39

RI, retention index; t, trace, <0.05.

4. Discussion and Conclusion

The loss of total oil is parallel with the changes of the absolute amount of each compound, but there are differences in the change of the relative amounts of the compounds because of different sensitivity for temperature. At high temperatures, the biological structure of the oil glands of medicinal and aromatic plants can be affected and the epithelial cells in the dried samples of some sensible plants could collapse [9, 31]. Drying may have a role in total or partial loss of essential oil [13]. Alteration on chemical composition of essential oils may be related to the connection between variations in temperature with plants metabolic activity. The effect of shade and sun drying resulted in the loss of methyl eugenol, α -pinene, α -thujene and γ -terpinene. These components seem to have more affinity to the water fraction contained in bay leaves and thereby, they were lost with water during drying process. On the other hand, sun drying method had a stimulative

effect on some other compounds biosynthesis and accumulation such as α -phellandrene, limonene and 1,8 cineole.

The chemical composition of essential oil of aromatic plants depends on the place of origin [32-35] and plant growth stage [36-41]. Some reports on the essential oil composition of *E. tenuifolia* subsp. *sibthorpiana* from various locations were previously published. Akgül and Chialva [2], reported the main components as α -phellandrene (51%), methyl eugenol (25%), d-3-carene (5.7%), β -phellandrene (5%) and *p*-cymene (4.3 %). Baser et al. (1994) reported that the oil contained over 30 components and composed of 52% α -phellandrene, 18% methyl eugenol and 15% *p*-cymene. Georgiou et al. [42] found that the essential oil contained 28 compounds and the main components of the oil were α -phellandrene (43.8%) and methyl eugenol (28.6%). However, some researches stated that methyl eugenol content of essential oil was higher than that

of α -phellandrene. Telci and Hisil [6], reported 45 components in essential oil of the same species and methyl eugenol (% 52.4-62.9), α -phellandrene (% 30.4), p-cymene (% 7.8-9.1) and δ -3-carene (% 3.3-5.7) were the main components of the oil. Baser et al. [17], reported that the main component of oil was methyl eugenol (58.7%), other important compounds were α -phellandrene (30.4%), p-cymene (7.8-9.1%) and d-3-carene (3.3-5.7%). Ozcan et al. [43] determined methyl eugenol (36.6%), d-3-carene (36.6%), p-cymene (7.6%), and α -phellandrene (6.1%) in the essential oil from plants collected in Turkey. Constituents of essential oils from *E. tenuifolia* subsp. *sibthorpiana* collected on April, May and June were δ -3-carene (36.98%, 38.8% and 30.01%), methyl eugenol (21.10%, 25.04% and 25.96%), α -phellandrene (14.50%, 21.71% and 29.26%), p-cymene (5.08%, 2.01% and 1.96%) and β -phellandrene (3.11%, 3.47% and 4.70%), respectively [19].

Detected components of the essential oil of *E. tenuifolia* subsp. *sibthorpiana* varied between 21 to 45. However; the major constituents were α -phellandrene and methyl eugenol. Some other components such as; δ -3-carene, β -phellandrene reported on other papers were not detected in this study. These results suggest that *E. tenuifolia* subsp. *sibthorpiana* populations may have different chemotypes. It can be concluded that the variation in the essential oil content and composition of essential oil bearing plants depends on different stages of plant growth, as well as plant parts and drying methods. Over all, research results indicated that there were considerable differences in the content and composition of the essential oil of *E. tenuifolia* subsp. *sibthorpiana* at different growth stages and drying methods. It is believed that these differences are associated with modifications in secondary metabolism along with the growth and development of the plant.

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