EFFECTS OF REAPING TIME ON VOLATILE COMPONENTS OF NATURAL *PHLOMIS RIGIDA* LABILL. AND *PHLOMIS MONOCEPHALA* P.H.DAVIS IN TURKEY

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Abstract. In this study, conducted between 2015 and 2017, 49 different volatile components were identified from *Phlomis rigida* Labill that was reaped from Gölcük highland located on Konya Seydişehir-Bozkır road and *Phlomis monocephala* P.H.Davis that was reaped from Mersin Silifke Bahçederesi Village in Turkey at three different periods (pre-flowering, flowering and post-flowering) through gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). The main components of *Phlomis rigida* were found to be (E)-2-Hexenal (9.21%), β-Caryophyllene (60.23%) and Germacrene D (9.76%). The main components of *Phlomis monocephala* were found to be α-Pinene (15.59%), (E)-β-Farnesene (17.69%) and Germacrene D (18.92%). It was concluded that reaping *P. rigida* and *P. monocephala* species during the flowering period was important for the yield of volatile components.
Keywords: *Phlomis, GC-MS, Germacrene D, α-Pinene, Turkey*

Introduction

Turkey has a rich diversity of plant and animal species thanks to its geographical location, geological, geomorphological and climatic features. Turkey is a bridge between Asia and Europe; therefore, it has three different flora zones. For that reason, several species of Asian and European origin are distributed in Turkey (Durmuşkahya, 2005). Located at the intersection of three phytogeographical zones, Turkey has a high species endemism due to the high species richness and endemism by ecological and phytogeographical differentiation as a result of several factors such as its function as a bridge between the floras of South Europe and Southeast Asia, and since Anatolia is the hub of origin and differentiation for several genera and sections (Tan, 1992). Flora of Turkey has around 11,466 plant taxa, whereas European continent has around 12,000 plant taxa. Almost 3649 plant taxa out of those that are naturally distributed in Turkey are endemic (Güner et al., 2012). Hundreds of plant species naturally distributed in Turkey including the endemic species have a very high medical and aromatic value (Baydar, 2009).

Volatile oils have been used as therapeutic medicine since ancient times (Kubecka, 1973). Volatile oils are volatile lipoid mixtures with strong smell that are derived from plants or herbal drugs through water or steam distillation, and that are liquid under normal conditions but sometimes can be frozen (Tanker and Tanker, 1990). In particular, those medical and aromatic plants with a rich content of volatile oil are especially important. Volatile oils (extracts, etheric oils) and their aromatic extracts are

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commonly used in the fragrance and flavour industries as the source for perfumes, food additives, cleaning substances, cosmetic and medicine preparations, aroma-chemicals or initial material for synthesis of nature-identical and semi-synthetic beneficial aroma chemicals. Particularly, there has been a tremendous increase in the demand for volatile oils to be used in aromatherapy practices that have become popular in recent years (Weiss, 1997).

Across the world, Lamiaceae family that can grow nearly everywhere regardless of habitat type and elevation is one of the largest families considering the number of its members (Watson and Dallwitz, 1978). Its widest distribution area is located in the Mediterranean Basin, while the taxa in this family are mainly the open field plants except some of the genera growing in the tropical rain forests (Watson and Dallwitz, 1978; Morgaris et al., 1982; Davis, 1988). With 200 genera and around 3200 species, Lamiaceae family has 45 genera and 546 species in Turkey (Davis, 1982; Baytop, 1997).

Phlomis that is one of the genera of Lamiaceae family has around 100 species around the world. It is represented by a total of 52 taxa including 39 taxa and 13 hybrids in The checklist of the Flora of Turkey (Güner et al., 2012). Its appetising leaves and flowers are used as anti-allergic, diuretic, anti-diarrheal, gas relieving, stomach relieving, pain-killing, antidiabetic herbal tea and tonic. Furthermore, it s known to be commonly used by people for respiratory diseases and haemorrhoid (Harput et al., 2006).

In the last decade, there have been important developments with respect to the discovery of pharmacological mechanisms of *Phlomis* species and associated different components. The phytochemical studies on *Phlomis* showed that the species contained iridoid, flavonoid, phenylpropanoid, phenylethanoid, lignan, neolignan, diterpenoid, alkoloid and volatile oils (Kamel et al., 2000; Zhang and Wan, 2008).

Phlomis rigida Labill. is a perennial herbaceous plant that can grow as high as 125 cm with glandular hairs. Its leaves are large, oblong, elliptic or cuneate and have a size of 5-30 x 2-10 cm with pale greenish colour and tomentose hairs. It is verticillate, has 5-8- 18 flowers, numerous bracteoles, subulate 20-25 mm, calyx is 15-23 mm and dense hispid stellate-tomentose hairs, corolla is pink-purple. On the other hand, *Phlomis monocephala* P.H.Davis is a bush species that can grow as high as 150 cm with glandular hairs. Its leaves are ovate-lanceolate or ovate-oblong, cuneate or truncate, under leaf surface is yellowish and densely stellate-tomentose, upper hairs are greenish stellate-tomentose, and its size is around 2-6,5 x 1-3,5 cm. It is verticillate with 6-12 flowers, bracteole lanceolate is 5-8x1,5-2 mm long, calyx is 10-14 mm and has stellate-tomentose-lanate hairs, corolla is yellow (Davis, 1982). The purpose of this study was to identify the impact of different reaping times of *Phlomis rigida* and *P. monocephala* taxa on volatile oil components and determine the appropriate reaping time.

Materials and Methods

This study was conducted between 2015 and 2017. The research materials that were *Phlomis rigida* Labill. and *Phlomis monocephala* P.H.Davis were reaped from Gölcük Highland located on Konya Seydişehir-Bozkır road at an elevation of 1732 m (37°13´21´´N, 32°00´28´´E) and Mersin Silifke Bahçederesi Village in Turkey at an elevation of 527 m (36°21´17´´N, 33°48´28´´E), respectively (*Figure 1*). The samples were collected from the pre-determined sampling plots in 3 different periods, which were pre-flowering, flowering and post-flowering periods.

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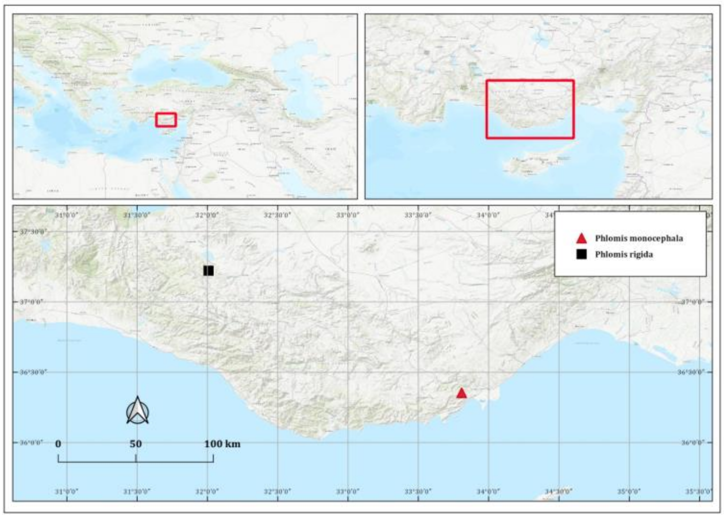

Figure 1. *Stands of collecting samples*

The leaf and flower samples of *Phlomis rigida* and *P. monocephala* collected from the sampling plots were placed in paper packages and transported to the laboratory without delay and avoiding their exposure to sunlight by private car in same day.

Flower and leaf samples were dried in room temperature (25ºC) and processed for solid phase microextraction (SPME). Samples were put into 10 mL vials from each after incubation at 60°C during 30 min. Then, GC-MS (Shimadzu 2010 Plus) process were applied at a temperature of 250°C for desorption (5 min) of the adsorbed volatile compounds for analysis. Constituents were identified by using libraries.

Results

The volatile components in the leaves and flowers of *Phlomis rigida* Labill. and *P. monocephala* P.H.Davis collected from the sampling plots were identified through gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME).

SPME analysis revealed 49 different volatile components *Phlomis r*igida and *P. monocephala*. The results of these samples are presented in *Table 1*.

(E)-2-Hexenal, β-Caryophyllene and Germacrene D were found to be the main components in *Phlomis rigida*. The rates in the pre-flowering period were as follows: (E)-2-Hexenal (8.23%), β-Caryophyllene (46.65%) and Germacrene D (8.74%); while the rates during the flowering period were as follows: (E)-2-Hexenal (9.21%), β-Caryophyllene (60.23%) and Germacrene D (9.76%); and the rates in the post-flowering period were as follows: (E)-2-Hexenal (8.85%), β-Caryophyllene (56.01%) and Germacrene D (9.29%) (*Table 1*).

α-Pinene, (E)-β-Farnesene and Germacrene D were found to be the main components of *Phlomis monocephala*. The rates in the pre-flowering period were as follows: α-Pinene (14.90%), (E)-β-Farnesene (16.47%) and Germacrene D (17.78%); while the rates during the flowering period were as follows: α-Pinene (15.59%), (E)-β-Farnesene (17.69%) and Germacrene D (18.92%); and the rates in the post-flowering period were as follows: α-Pinene (14.63%), (E)-β-Farnesene (15.69%) and Germacrene D (16.16%) (*Table 1*).

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Table 1. *Volatile components of Phlomis rigida Labill. and P. monocephala P.H.Davis in different vegetation periods*

			P. rigida			**P. monocephala**		
	Rt	**Components**	**Pre flowering**	**Flowering**	**Post flowering**	**Pre flowering**	**Flowering**	**Post flowering**
1.	1.403	Dimethyl sulphide	1.04	0.44	0.67	-	-	-
2.	1.440	2-Methyl-propenal	0.35	0.18	0.29	-	-	-
3.	1.517	3-Methyl-2-butanone	0.30	0.10	0.19	-	-	-
4.	1.887	Crotonaldehyde	0.37	0.23	0.39	0.77	0.80	0.58
5.	1.929	3-Methylbutanal	1.16	0.21	0.46	0.37	0.10	0.84
6.	2.009	2-Methylbutanal	1.08	0.33	0.85	0.06	0.10	0.09
7.	2.197	Ethyl vinyl ketone	0.32	0.09	0.15	-	-	-
8.	2.325	Pentanal	0.51	0.40	2.05	0.45	1.27	0.76
9.	2.357	Furan, 2-ethyl-	3.38	0.63	0.13	-	-	0.32
10.	3.181	(E)-2-Pentenal	0.30	0.12	0.30	0.25	0.73	0.62
11.	4.101	n-Hexanal	2.52	2.53	2.93	1.97	0.77	3.88
12.	5.514	(E)-2-Hexenal	8.23	9.21	8.85	0.78	1.40	1.85
13.	5.589	cis-3-Hexene-1-ol	1.71	0.34	1.13	0.11	0.13	0.35
14.	6.028	n-Hexanol	0.25	0.16	0.20	0.15	0.11	0.09
15.	7.025	Heptanal	1.62	0.91	0.82	0.15	0.39	0.24
16.	7.837	α-Thujene	0.20	0.17	0.20	0.18	1.01	1.73
17.	8.069	α-Pinene	1.37	0.56	0.86	14.90	15.59	14.63
18.	8.944	2-Heptenal	0.31	0.34	0.10	0.67	0.15	0.79
19.	9.043	Benzaldehyde	1.31	0.74	1.03	0.50	0.17	0.40
20.	9.647	2-.β.-Pinene	0.76	0.30	0.49	0.60	0.59	0.50
21.	9.909	Vinyl amyl carbinol	2.00	1.09	0.51	0.50	0.36	0.35
22.	10.023	6-Methyl-5-hepten-2-one	1.18	0.32	0.49	-	-	-
23.	10.202	Myrcene	0.65	0.20	0.26	1.86	1.20	1.07
24.	10.208	2-Pentylfuran	0.50	0.57	0.29	-	-	-
25.	10.527	2-[(2E)-2-Pentenyl]furan	0.31	0.41	0.30	-	-	-
26.	10.705	Octanal	0.47	0.40	0.21	0.41	0.18	0.78
27.	10744	α-Phellandrene	-	-	-	0.56	0.28	0.18
28.	10.968	2,4-Heptadienal	0.42	0.09	0.22	-	-	-
29.	11.458	Cymene	-	-	-	0.33	0.34	0.60
30.	11.649	Limonene	1.89	1.00	1.28	2.70	2.36	3.65
31.	12.005	3-Octen-2-one	0.09	0.10	0.20	6.79	6.97	5.72
32.	12.134	Benzeneacetaldehyde	0.57	0.38	0.55	-	-	-
33.	12,370	Trans-.β.-Ocimene	-	-	-	0.68	0.42	0.11
34.	12.763	(E)-2-Octenal	0.20	0.28	0.55	0.45	0.32	0.58
35.	14.417	Linalool	0.97	0.57	0.62	-	-	-
36.	14.590	n-Nonanal	0.65	0.98	0.52	0.70	0.29	1.24
37.	15.501	(4E,6Z)-Alloocimene	-	-	-	0.73	0.82	0.23
38.	17.824	Methyl salicylate	0.30	0.28	0.19	-	-	-
39.	18.452	Decanal	0.70	0.41	0.16	1.02	0.38	0.93
40.	22.975	Cyclohexane	-	-	-	0.22	0.23	0.17
41.	23.086	δ-Elemene	-	-	-	0.41	0.43	0.21
42.	23.506	.α.-Cubebene	0.23	0.22	0.16	5.16	5.13	5.38
43.	24.264	Ylangene	-	-	-	0.45	0.70	0.34
44.	24.479	.α.-Copaene	0.66	0.70	0.55	1.93	1.77	1.21
45.	24.744	.β.-Bourbonene	0.39	0.23	0.15	0.25	1.97	0.24
46.	24.949	.β.-Elemene	-	-	-	0.23	0.68	0.15
47.	25.546	.α.-Gurjunene	-	-	-	0.34	0.48	0.36
48.	26.076	β-Caryophyllene	46.65	60.23	56.01	1.07	1.01	1.26
49.	26.238	10,10-dimethyl-2,6-bis(methylene)-	0.43	0.44	0.25	0.30	0.50	0.70
50.	26.565	γ.-Muurolene	-	-	-	1.56	1.84	1.76
51.	26.570	(+)-Aromadendrene	0.26	0.17	0.51	-	-	-
52.	27.108	(E)- β-Farnesene	-	-	-	16.47	17.69	15.69
53.	27.124	.α.-Humulene	1.50	1.25	1.55	-	-	-
54.	27.359	Epi-bicyclosesquiphellandrene	-	-	-	0.30	0.52	0.50
55.	27.730	Cadina-1(6),4-diene	-	-	-	0.46	0.36	0.94
56.	27.971	Germacrene-D	8.74	9.76	9.29	17.78	18.92	16.16
57.	28.305	α.-Copaene	0.40	0.23	0.29	2.50	1.57	2.76
58.	28.438	Bicyclogermacrene	1.09	0.62	0.74	3.46	3.04	3.17
59.	28.548	α-Muurolene	0.21	0.17	0.50	0.25	0.62	0.20
60.	28.998	.γ.-Cadinene	0.39	0.21	0.50	2.80	0.41	0.71
61.	29.176	δ-Cadinene	0.73	0.40	0.54	3.83	4.27	3.62
62.	30.409	Germacrene B	-	-	-	1.38	0.38	1.08
63.	30.954	Spathulenol	-	-	-	0.21	0.25	0.28
64.	31.105	Caryophyllene oxide	0.33	0.30	0.52	-	-	-

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Discussion and Conclusions

SPME analysis revealed that *Phlomis rigida* Labill. and *P. monocephala* P.H.Davis had 49 different volatile components. Main components of *Phlomis rigida* were (E)-2-Hexenal (9.21%), β-Caryophyllene (60.23%) and Germacrene D (9.76%). Demirci et al. (2006) found through GC-MS analysis that the main components of *Phlomis rigida* Labill. were β-Caryophyllene (31.2% - 38.7%) and β-selinene (13.1% - 15.1%). β-Caryophyllene was found to be the dominant component in that study. This finding is consistent with our result. Contrary to the abovementioned study, (E)-2-Hexenal and Germacrene D were also identified as dominant components in our study.

α-Pinene (15.59%), (E)-β-Farnesene (17.69%) and Germacrene D (18.92%) were found to be the main components of *Phlomis monocephala*. In a study conducted by Demirci et al. (2009), they aimed at exploring the antimicrobial effects and phytochemical profile of *Phlomis lunariifolia* Sm., *Phlomis amanica* Vierh., *Phlomis monocephala* P.H. Davis, *Phlomis sieheana* Rech. fil., *Phlomis armeniaca* Willd. species in Turkey. They applied GC and MS for the analysis of the volatile oils that they derived through hydrodistillation. They identified 143 components in the volatile oils of *Phlomis amanica* Vierh. and *Phlomis monocephala* P.H.Davis. They found that 8(14), 15-Isopimaradien-11α-ol (1) were the main components in the volatile oils of *Phlomis monocephala* P.H.Davis. This finding is different from our result. Contrary to that study, Germacrene D, (E)-β-Farnesene and α-Pinene were identified as the dominant components in our study.

In conclusion, the volatile components β-Caryophyllene, (E)-2-Hexenal, Germacrene D, (E)-β-Farnesene and α-Pinene obtained in this study may be considered as potential sources and materials for the pharmacology and cosmetic industry thanks to their antimicrobial activities. It was found that reaping *P. rigida* and *P. monocephala* species, which were collected from the field in the form of leaves and flowers, during the flowering period was important with respect to the yield of volatile components. These findings are considered to help preventing the haphazard collection of plants by traders and local people and economic losses that may arise due to misinformation and raise awareness for the collection of the plants. Although *Phlomis* taxa are used in many areas, there are limited numbers of studies conducted on *Phlomis* taxa in Turkey. There is a need for further studies in this field.

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