NEW DEVELOPMENT ON MEDICINAL AND AROMATIC PLANTS

EDITED BY
Assist. Prof. Dr. Gülen ÖZYAZICI

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PREFACE

Anatolia is a biodiversity hotspot and rich in plants species due to climatic, geographic and cultural diversity. Medicinal and aromatic plants are natural resources for health care and perfumery since antiquity. They are one step beyond the basic instinct of human; eating. Demand for herbs and aromatic products is increasing every year worldwide. Quality is an important issue in the production and utilization of medicinal and aromatic plants. Stress is an important trigger for production of secondary substances and minor components, helps to increase content and modify chemical balances in mixtures.

Cropping species outside the adopted ecology is a stress factor. Also intra and inner-species diversity is very high in medicinal and aromatic plants which may help to utilise these crops in abiotic stressed soils. A series of research including production, analyses, antioxidant activities and dye properties of medicinal plants, their use and utilization in alternative areas, and the determination of the chemical components of different species are included in this book under the name of "NEW DEVELOPMENT ON MEDICINAL AND AROMATIC PLANTS" in order to contribute to this process. I would like to thank the respected and valuable scientists who have contributed to this book, which includes new and up-to-date data, and I pay my respects with the hope that the work will be useful for the scientific world.

Assist. Prof. Dr. Gülen ÖZYAZICI
EDITOR

CHAPTER 1

EXAMINATION OF MEDICINAL AND AROMATIC PLANTS CULTIVATED IN TURKEY IN TERMS OF YEARS, REGIONS AND PROVINCES

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INTRODUCTION

The use of medicinal and aromatic plants in disease treatment is as old as human history. Our ancestors have always used the natural substances they could find in their environment to heal. This approach has brought the treatment with herbs to the present day, and has made 80% of the world population trust medicinal plants today (Mathe et al., 2015). However, when the developments in the production and use of medicinal and aromatic plants in the twentieth century are examined, the innovations brought by technology and social and political changes at the beginning of the century caused the use of herbs as medicine to decrease rapidly. The synthesis of organic chemicals in the 1930s and 1940s encouraged the production of synthetic drugs in addition to medicinal plants. Economic and social changes following World War II and new definitions of plants and treatments caused a decrease in the use of plant extracts and plants until the end of the 1970s in western countries, which modernized with industrial advances as a result of the acquisition of synthetic chemical drugs (Craker et al., 2003; Faydaoğlu & Sürücüoğlu, 2011). Since the 1980s, people's awareness of health has increased and their desire not to be exposed to the effects of chemicals has increased the demand for natural and organic products and brought the treatment with herbs on the agenda (Göktaş & Gıdık, 2019). In addition, the side effects of synthetic and chemical-containing drugs on human health has been another reason for the orientation to medicinal plants. As a result, medicinal and aromatic plants have become a rapidly growing market in the world (Bayraktar et al., 2017). Turkey has different vegetations and rich floristic diversity due to its

geographical location, geographical structure, soil types and climate factors (Yıldıztekin et al., 2019). There are a total of 422,000 plant species in the world, 52,885 of which are used for medicinal and aromatic purposes. The highest number of medicinal and aromatic plant species was detected in China with 4,941, followed by India with 3,000, USA with 2,564, Vietnam with 1,800, Malaysia with 1200 species and Indonesia with 1,000 species. The number of medicinal and aromatic plant species in Turkey is 500 (Temel et al., 2018). A significant part of medicinal and aromatic plants traded in our country are collected from nature, but there are also species that are cultured (Özyazıcı, 2019). In this study, the sowing-planting areas and production amounts of medicinal and aromatic plants that are cultivated in our country and contribute to the economy were examined in years, regions and provinces.

1. MATERIAL AND METHOD

In the study, sage, anise, black cumin, rose, poppy, nettle, thyme, red pepper, cumin, coriander, lavender, blueberry, lemon balm, mint, heather and hops, which have an important place in medicinal and aromatic plant trade, tables were prepared on the basis of five years of production data from Turkey Statistical Institute (TURKSTAT) obtained by years, regions and provinces; and, production areas and production quantities were investigated.

2. RESULTS

2.1. Sage

When the regions are evaluated according to the five-year total production area and production amount in sage production, it is seen that Aegean Region takes the first place with 16.787 decares and 1.936 tons. The Aegean Region was followed by the Mediterranean Region with 5,529 decares and 1,731 tons of production, and the Marmara Region with 193 tons of production on an area of 1.419 decares. The least sowing-planting area and production amount of sage was in the Southeast Region with a yield of 2 tons in an area of 12 decares (Table 1). Planting area and production amount (4.123 decares/557 tons), which increased in sage production in 2017, decreased slightly in 2018 (3.951 da/428 tons), but in the following years both data increased. Sage production area (6,655 da) and yield (1,271 tons) reached the highest level in 2020 compared to the other four years. The Aegean Region, which had the highest planting area in 2016 (3.496 da/390 tons), 2017 (3.755 da/502 tons) and 2018 (3.516 da/367 tons), fell behind the

Mediterranean Region in terms of yield in 2019 (2.566 da) and 2020 (2.778 da) with the increase in sage cultivation in the Mediterranean Region even though the production area was higher. When the provinces with cultivation are examined, Denizli and Antalya stand out in terms of production area and production amount. In Table 1, it is seen that sage cultivation started for the first time in 2020 in Burdur, Hatay, Şanlıurfa and Ankara, and although the production has been made in 3 decares of area in Kayseri in the last four years, the yield in tons has not been recorded.

Table 1. Sage production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Adana	-	-	85	13	85	13	10	4	10	4
	Antalya	5	1	5	1	5	1	2.556	883	2.056	669
	Burdur	-	-	-	-	-	-	-	-	710	142
	Hatay	-	-	-	-	-	-	-	-	2	0
Total Production Area / Yield		**5**	**1**	**90**	**14**	**90**	**14**	**2.566**	**887**	**2.778**	**815**
5-Year Total Production Area: 5.529 da											
5-Year Total Yield:	**1.731 tons**										
The Aegean Region	Denizli	2.735	275	2.781	273	2.611	246	1.637	157	2.120	207
	Kütahya	641	97	645	181	491	62	541	70	602	78
	Manisa	-	-	185	28	185	28	185	28	181	27
	Muğla	114	17	118	17	203	28	209	29	493	75
	Uşak	-	-	20	2	20	2	20	2	20	2
	Izmir	6	1	6	1	6	1	6	1	6	1
Total Production Area / Yield		**3.496**	**390**	**3.755**	**502**	**3.516**	**367**	**2.598**	**287**	**3.422**	**390**
5-Year Total Production Area:16.787 da											
5-Year Total Yield:	**1.936 tons**										
The Southeastern Anatolia Region	Şanlıurfa	-	-	-	-	-	-	-	-	12	2
Total Production Area / Yield		**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**12**	**2**
5-Year Total Production Area: 12 da											
5-Year Total Yield:	**2 ton**										
The Central Anatolia Region	Ankara	-	-	-	-	-	-	-	-	14	4
	Eskişehir	5	1	5	1	5	1	5	1	22	4
	Karaman	35	5	30	4	27	4	23	3	-	-
	Kayseri	-	-	3	0	3	0	3	0	3	0
Total Production Area / Yield		**40**	**6**	**38**	**5**	**35**	**5**	**31**	**4**	**39**	**8**
5-Year Total Production Area: 183 da											
5-Year Total Yield:	**28 tons**										
The Black Sea Region	Düzce	-	-	-	-	-	-	45	5	37	5
Total Production Area / Yield		**-**	**-**	**-**	**-**	**-**	**-**	**45**	**5**	**37**	**5**
5-Year Total Production Area: 82 da											
5-Year Total Yield:	**10 tons**										
The Marmara Region	Tekirdağ	140	14	240	36	310	42	362	50	367	51
Total Production Area / Yield		**140**	**14**	**240**	**36**	**310**	**42**	**362**	**50**	**367**	**51**
5-Year Total Production Area:1.419 da											
5-Year Total Yield:	**193 tons**										
Total by Years Production Area / Yield		**3.681**	**411**	**4.123**	**557**	**3.951**	**428**	**5.602**	**1.233**	**6.655**	**1.271**

2.2. Anise

As can be seen in Table 2, according to the total data of the last five years, the highest cultivation was done in the Mediterranean Region with 301.562 decares and in the Aegean Region with 270.985 decares, but the highest yield was obtained in the Aegean Region with 20.774 tons. The Mediterranean Region took the second place with 20,436 tons. Central Anatolia Region (185.081 da/12.264 tons) and Marmara Region (19.700 da /1.434 tons) take the third and fourth place in terms of decare and yield. When the data of the last five years of anise cultivation in the Mediterranean, Aegean, Central Anatolia and Marmara Regions are examined separately in Table 2, it is seen that there are significant changes in terms of production area by years. The cultivation area, which was 136.552 decares in 2016, decreased in 2017 (121.833 decares), increased in 2018 (124.455 decares) and 2019 (239.171 decares), but decreased again in 2020 (155.317 decares). It is seen that the same situation arises in terms of yield. The highest production area (239,171 da) and yield (17,589 tons) were reached in 2019. When the cultivation areas and yields of the regions are examined by years, the Mediterranean Region has the highest values in 2016 (71.640 da/4.810 tons), 2017 (65.992 da/4.242 tons) and 2018 (63.299 da/4.129 tons). However, the Central Anatolia Region outperformed the Mediterranean Region with a yield of 7.447 tons on an area of 104.089 decares in 2019; and, the Aegean outperformed the Mediterranean Region region with a yield of 4.549 tons on an area of 64.968 decares in 2020. Burdur ranked first in terms of both cultivation area and yield

in 2016 (60.840 da/3.927 tons), 2017 (55.392 da/3.371 tons), 2018 (53.999 da/3.432 tons), but in 2019, Konya took the first place from Burdur with a yield of 5.339 tons on an area of 70.569 da. Although Denizli has the highest production area (29.812 da) in 2020, it ranks second after Burdur (1.891 tons) in terms of yield (1.849 tons). It is seen that anise production started in Kırıkkale in 2019 and in Aksaray, Kırşehir and Sivas in 2020.

2.3. Nigella

According to TURKSTAT 2020 data, while the cumin cultivation in all regions of Turkey was carried out in the years between 2016 to 2020, in terms of total production area and amount, the Aegean Region has stood out (43 953 in/4,274 tons) and was followed by the Mediterranean region (37 981 in/3,723 tons) (Table 3).

Although the production area and production amount increased until 2020, it decreased to 33.773 decares in 2020 and the yield decreased to 3.412 tons. When five-year values of 2016-2020 in terms of regions of Nigella cultivation were examined, it was determined that cultivation was done in all regions; Central Anatolia region was first with 56,066 decars and 5.778 tons of yield, and Aegean region was second with 43,953 decars and 4.274 tons. When the provinces where Nigella cultivation is carried out are examined, it is seen that Konya takes the first place in terms of production area and production amount in 2016 (10.091 decares/1.030 tons) and 2017 (10.179 decares/882 tons). However, in the following years, the ranking changed, Burdur in 2018

(9.883 decares/923 tons) and 2019 (11.318 decares/929 tons) and Uşak (10.750 decares/1.170 tons) in 2020 took the first place. In Table 3, it is seen that Nigella farming started in Hatay, Gaziantep and Tokat in 2020.

Table 2. Anise production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Adana	-	-	-	-	-	-	51	3	-	-
	Antalya	10.800	883	10.600	871	9.300	697	10.300	904	11.650	974
	Burdur	60.840	3.927	55.392	3.371	53.999	3.432	53.268	3.483	25.362	1.891
Total Production Area / Yield		**71.640**	**4.810**	**65.992**	**4.242**	**63.299**	**4.129**	**63.619**	**4.390**	**37.012**	**2.865**
5-Year Total Production Area: 301.562 da											
5-Year Total Yield: 20.436 tons											
The Aegean Region	Afyonkarahisar	7.219	540	6.367	545	11.064	937	20.652	1.664	19.371	1.321
	Denizli	35.415	2.387	24.371	1.722	21.863	1.464	29.301	2.167	29.812	1.849
	Kütahya	43	3	42	2	10	1	-	-	-	-
	Muğla	9.500	950	9.600	960	10.000	1.000	8.000	880	4.000	440
	Uşak	300	27	341	28	884	69	10.735	837	11.750	937
	Izmir	150	5	70	3	50	2	40	2	35	2
Total Production Area / Yield		**52.627**	**3.912**	**40.791**	**3.260**	**43.871**	**3.473**	**68.728**	**5.550**	**64.968**	**4.549**
5-Year Total Production Area: 270.985 da											
5-Year Total Yield: 20.744 tons											
The Central Anatolia Region	Aksaray	-	-	-	-	-	-	-	-	100	7
	Ankara	35	2	1.128	79	3.384	232	29.162	1.761	27.135	1.349
	Eskişehir	700	49	935	63	1.123	82	4.265	341	2.128	149
	Konya	4.600	204	6.652	319	9.728	525	70.569	5.339	21.955	1.651
	Kırıkkale	-	-	-	-	-	-	93	6	630	42
	Kırşehir	-	-	-	-	-	-	-	-	341	22
	Sivas	-	-	-	-	-	-	-	-	418	42
Total Production Area / Yield		**5.335**	**255**	**8.715**	**461**	**14.235**	**839**	**104.089**	**7.447**	**52.707**	**3.262**
5-Year Total Production Area: 185.081 da											
5-Year Total Yield: 12.264 tons											
The Marmara Region	Balıkesir	1.000	85	600	42	500	35	500	35	450	27
	Bursa	5.950	429	5.735	413	2.550	188	2.235	167	180	13
Total Production Area / Yield		**6.950**	**514**	**6.335**	**455**	**3.050**	**223**	**2.735**	**202**	**630**	**40**
5-Year Total Production Area: 19.700 da											
5-Year Total Yield: 1.434 tons											
Total by Years Production Area / Yield		**136.552**	**9.491**	**121.833**	**8.418**	**124.455**	**8.664**	**239.171**	**17.589**	**155.317**	**10.716**

Table 3. Nigella production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
	Adana	-	-	139	28	-	-	-	-	-	-
The	Antalya	250	43	1.050	149	865	136	1.340	203	1.240	91
Mediterranean	Burdur	280	28	308	31	9.883	923	11.318	929	9.838	992
Region	Hatay	-	-	-	-	-	-	-	-	18	3
	Isparta	350	39	230	26	288	31	268	29	195	23
	Kahramanmaraş	4	0	10	2	97	15	10	2	-	-
Total Production Area / Yield		**884**	**110**	**1.737**	**236**	**11.133**	**1.105**	**12.936**	**1.163**	**11.291**	**1.109**
5-Year Total Production Area: 37.981 da											
5-Year Total Yield: 3.723 tons											
The East	Kars	-	-	-	-	-	-	476	67	-	-
Anatolia	Malatya	-	-	-	-	37	4	32	3	-	-
Region											
Total Production Area / Yield		**-**	**-**	**-**	**-**	**37**	**4**	**508**	**70**	**-**	**-**
5-Year Total Production Area: 545 da											
5-Year Total Yield: 74 tons											
	Afyonkarahisar	37	6	70	11	92	14	138	22	168	31
The Aegean	Denizli	-	-	-	-	10	2	23	4	25	4
Region	Kütahya	1.731	206	2.092	207	1.481	153	910	93	758	75
	Muğla	-	-	-	-	5	1	2	0	4	0
	Uşak	3.550	378	6.581	542	6.791	576	8.735	779	10.750	1.170
Total Production Area / Yield		**5.318**	**590**	**8.743**	**760**	**8.379**	**746**	**9.808**	**898**	**11.705**	**1.280**
5-Year Total Production Area: 43.953 da											
5-Year Total Yield: 4.274 tons											
The	Gaziantep	-	-	-	-	-	-	-	-	100	19
Southeastern	Kilis	-	-	-	-	27	4	31	5	-	-
Anatolia Region											
Total Production Area / Yield		**-**	**-**	**-**	**-**	**27**	**4**	**31**	**5**	**100**	**19**
5-Year Total Production Area: 158 da											
5-Year Total Yield: 28 tons											
	Ankara	1.455	223	2.565	277	1.320	106	510	65	785	117
	Eskişehir	5	1	464	44	240	19	450	45	326	24
	Kayseri	200	34	200	32	750	72	550	70	455	68
The Central	Konya	10.091	1.030	10.179	882	5.550	626	6.431	753	2.334	219
Anatolia	Kırıkkale	-	-	85	17	50	9	-	-	-	-
Region	Kırşehir	950	123	150	14	118	11	50	5	120	12
	Nevşehir	20	2	12	1	40	5	126	14	278	32
	Niğde	150	20	-	-	-	-	-	-	-	-
	Sivas	805	70	2.215	219	795	78	761	77	236	23
	Yozgat	529	62	1.409	139	900	63	100	7	1.307	68
Total Production Area / Yield		**14.205**	**1.565**	**17.279**	**1.625**	**9.763**	**989**	**8.978**	**1.036**	**5.841**	**563**
5-Year Total Production Area: 56.066 da											
5-Year Total Yield: 5.778 tons											
	Çorum	-	-	2.070	227	1.900	244	2.000	199	2.120	211
The Black Sea	Karabük	-	-	-	-	-	-	200	4	-	-
Region	Samsun	1.153	139	1.004	115	840	93	813	90	539	65
	Tokat	-	-	-	-	-	-	-	-	277	21
Total Production Area / Yield		**1.153**	**139**	**3.074**	**342**	**2.740**	**337**	**3.013**	**293**	**2.936**	**297**
5-Year Total Production Area: 12.916 da											
5-Year Total Yield: 1.408 tons											
The Marmara	Balıkesir	100	10	80	7	70	6	70	6	60	5
Region	Bursa	1.500	113	1.647	124	1.715	131	1.741	132	1.840	139
Total Production Area / Yield		**1.600**	**123**	**1.727**	**131**	**1.785**	**137**	**1.811**	**138**	**1.900**	**144**
5-Year Total Production Area: 8.823 da											
5-Year Total Yield: 673 tons											
Total by Years Production Area / Yield		**23.160**	**2.527**	**32.560**	**3.094**	**33.864**	**3.322**	**37.085**	**3.603**	**33.773**	**3.412**

2.4. Rose (oil)

According to Table 4, the oil rose production area and production amount in Turkey for the last five years have increased every year and reached 41,320 decars and 18,202 tons in 2020. As a result of the evaluation in terms of both the total area and total yield of five years of cultivation and of the years separately, it was determined that the most Nigella cultivation was made in the Mediterranean Region, and the Aegean Region ranked second. When the cultivation data by provinces are examined, it is seen that Isparta has the highest area and yield between 2016-2020, and Burdur takes the second place. It is seen that rose cultivation started in Kahramanmaraş in 2020, although it was cultivated in an area of 18 decares in Manisa in 2016, the yield in tons could not be obtained, and in Şanlıurfa, although it was cultivated in an area of 3 decares in 2019, cultivation was not continued in 2020 (Table 4).

Table 4. Rose (oil) production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Burdur	3.370	1.345	3.442	1.375	2.935	1.309	3.144	1.355	4.172	1.965
	Isparta	23.000	10.022	26.155	10.900	27.435	12.332	31.461	14.097	33.175	15.343
	Kahramanmaraş	-	-	-	-	-	-	-	-	40	2
Total Production Area / Yield		**26.370**	**11.367**	**29.597**	**12.275**	**30.370**	**13.641**	**34.605**	**15.452**	**37.387**	**17.310**
5-Year Total Production Area: 158.329 da											
5-Year Total Yield: 70.045 tons											
The Aegean Region	Afyonkarahisar	2.345	726	2.760	882	2.835	907	2.795	895	2.820	703
	Denizli	1.020	174	920	215	1.000	225	1.054	212	1.113	189
	Manisa	18	0	-	-	-	-	-	-	-	-
Total Production Area / Yield		**3.383**	**900**	**3.680**	**1.097**	**3.835**	**1.132**	**3.849**	**1.107**	**3.933**	**892**
5-Year Total Production Area: 18.680 da											
5-Year Total Yield: 5.128 tons											
The Southeastern Anatolia Region	Şanlıurfa	-	-	-	-	-	-	3	1	-	-
Total Production Area / Yield		-	-	-	-	-	-	**3**	**1**	-	-
5-Year Total Production Area: 3 da											
5-Year Total Yield: 1 tons											
Total by Years Production Area / Yield		**29.753**	**12.267**	**33.277**	**13.372**	**34.205**	**14.773**	**38.457**	**16.560**	**41.320**	**18.202**

2.5. Poppy (capsule)

In Turkey, which is accepted by the United Nations Organization as one of the legal producer countries, poppy cultivation is carried out in the Mediterranean region, Aegean Region, Central Anatolia region, Black Sea region and Marmara region (Table 5). When Table 5 is examined in terms of the five-year total production area and total yield of the regions, it is seen that the highest production area and yield are recorded in the Aegean Region (1,262,301 da/58,245 tons), and the Central Anatolia Region (492,457 da/30,725 tons) takes the second place. When the total cultivation areas and total yield amounts of poppy

cultivation of regions in 2016-2020 are examined, the Aegean Region has reached the highest values in both data every year, while the Central Anatolia Region has ranked second. On the basis of years, the cultivation area, which was 299,217 decares in 2016, decreased to 237,314 decares in 2017, increased in 2018 (451,226) and 2019 (677,369), and decreased again to 461,252 in 2020. Similar situation occurred in poppy yield. When the production area is evaluated in terms of provinces, Afyonkarahisar had the highest cultivation area in 2016-2020. In Konya, although cultivation was carried out in less land (61,384 da) in 2016, it surpassed Afyonkarahisar (63,744 da/4,586 tons) with a yield of 4,594 tons and the highest yields were obtained in Afyonkarahisar in other years.

2.6. Nettle

According to Table 6, nettle cultivation was carried out in an area of 5 da in Antalya in 2016, 2017 and 2018, and a yield of 1 ton was obtained. In Burdur, it was cultivated in 1 decare area in 2020, but yields in tons could not be obtained. When the data of the last five years are examined together, it is seen that 4 tons of production has been done in 16 decares.

Table 5. Poppy (capsule) production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Burdur	14.411	842	14.476	844	16.966	876	26.720	950	21.524	1.311
	Isparta	10.307	503	9.100	429	14.580	828	19.784	715	16.550	758
Total Production Area / Yield		**24.718**	**1.345**	**23.576**	**1.273**	**31.546**	**1.704**	**46.504**	**1.665**	**38.074**	**2.069**
5-Year Total Production Area: 164.418 da											
5-Year Total Yield:	**8.056 tons**										
The Aegean Region	Afyonkarahisar	63.744	4.586	68.257	4.667	121.074	8.475	160.848	7.613	119.009	6.005
	Denizli	43.211	1.650	26.157	1.484	73.674	3.622	127.735	5.066	78.994	2.893
	Kütahya	7.906	371	5.012	282	14.192	662	20.778	557	15.150	359
	Manisa	21.343	636	7.040	247	19.678	601	23.577	409	12.480	309
	Uşak	20.907	780	10.451	568	40.710	2.121	97.005	2.566	63.369	1.716
Total Production Area / Yield		**157.111**	**8.023**	**116.917**	**7.248**	**269.328**	**15.481**	**429.943**	**16.211**	**289.002**	**11.282**
5-Year Total Production Area: 1.262.301 da											
5-Year Total Yield:	**58.245 tons**										
The Central Region	Ankara	-	-	-	-	-	-	3.682	203	3.345	211
	Eskişehir	10.300	618	11.515	665	22.530	1.246	31.019	1.190	20.755	960
	Konya	61.384	4.594	63.563	3.758	90.155	6.973	103.492	5.626	70.717	4.681
Total Production Area / Yield		**71.684**	**5.212**	**75.078**	**4.423**	**112.685**	**8.219**	**138.193**	**7.019**	**94.817**	**5.852**
5-Year Total Production Area: 492.457 da											
5-Year Total Yield:	**30.725 tons**										
The Black Sea Region	Amasya	27.378	1.184	13.259	566	19.794	844	30.995	1.228	22.036	797
	Çorum	9.527	423	5.289	194	7.266	225	12.929	384	10.103	192
	Tokat	2.960	101	470	11	1.683	65	3.316	136	2.607	86
Total Production Area / Yield		**39.865**	**1.708**	**19.018**	**771**	**28.743**	**1.134**	**47.240**	**1.748**	**34.746**	**1.075**
5-Year Total Production Area: 169.612 da											
5-Year Total Yield:	**6.436 tons**										
The Marmara Region	Balıkesir	5.839	262	2.725	121	8.924	453	15.489	645	4.613	264
Total Production Area / Yield		**5.839**	**262**	**2.725**	**121**	**8.924**	**453**	**15.489**	**645**	**4.613**	**264**
5-Year Total Production Area: 37.590 da											
5-Year Total Yield:	**1.745 tons**										
Total by Years Production Area / Yield		**299.217**	**16.550**	**237.314**	**13.836**	**451.226**	**26.991**	**677.369**	**27.288**	**461.252**	**20.542**

Table 6. Nettle production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Antalya	5	1	5	1	5	1	-	-	-	-
	Burdur	-	-	-	-	-	-	-	-	1	0
Total Production Area / Yield		5	1	5	1	5	1	-	-	1	0
5-Year Total Production Area: 16 da											
5-Year Total Yield: 4 tons											

2.7. Thyme

Table 7 shows the five-year total sowing-planting area and production amount of the regions. In the Aegean Region, production was made on an area of 712,728 decares and a yield of 85,314 tons was obtained. In the Mediterranean Region, an area of 37,981 da and 3,723 tons of yield were recorded. According to the data of the last five years in thyme cultivation, the sowing-planting area and yield have increased every year, and the highest values were reached in 2020 (184,711 da/23,866 tons) (Table 7). In Table 7, it is seen that thyme cultivation in Turkey is carried out in all regions except the Eastern Anatolia Region. The Aegean Region surpassed other regions in terms of both production area and yield in 2016-2020. The Mediterranean Region took the second place. If the table is evaluated in terms of provinces, Denizli ranked first in terms of production area and yield in the years examined.

Table 7. Thyme production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
	Adana	-	-	-	-	-	-	5	1	5	1
	Antalya	184	32	460	68	470	70	663	142	688	264
The	Burdur	-	-	-	-	-	-	-	-	184	53
Mediterranean	Hatay	1.637	187	1.263	113	1.313	116	1.215	114	1.260	189
Region	Isparta	22	5	25	4	25	4	18	3	19	2
	Osmaniye	30	14	4	2	-	-	-	-	-	-
Total Production Area / Yield		**1.873**	**238**	**1.752**	**187**	**1.808**	**190**	**1.901**	**260**	**2.156**	**509**
5-Year Total Production Area: 9.490 da											
5-Year Total Yield: 1.384 tons											
	Afyonkarahisar	149	20	158	22	158	22	158	22	153	21
	Aydın	1100	165	1200	180	1.400	175	1500	188	1.915	246
	Denizli	111.152	12.624	111.769	12.487	129.095	14.009	145.280	15.729	172.461	21.324
The Aegean	Kütahya	2.029	475	1.940	409	1.921	288	1.721	254	1.369	177
Region	Manisa	2.320	828	2.120	712	2.120	724	2.140	733	2.285	755
	Muğla	255	48	273	49	276	48	292	53	310	70
	Uşak	2.026	262	2.110	393	2.110	396	3.575	670	3.813	710
	İzmir	60	24	-	-	-	-	-	-	15	2
Total Production Area / Yield		**119.091**	**14.446**	**119.570**	**14.252**	**137.080**	**15.662**	**154.666**	**17.649**	**182.321**	**23.305**
5-Year Total Production Area: 712.728 da											
5-Year Total Yield: 85.314 tons											
The Southeastern Region	Şanlıurfa	-	-	-	-	-	-	1	0	-	-
Total Production Area / Yield		-	-	-	-	-	-	**1**	**0**	-	-
5-Year Total Production Area: 1 da											
5-Year Total Yield: 0 tons											
	Ankara	-	-	-	-	-	-	-	-	12	2
The Central	Eskişehir	-	-	-	-	15	5	40	10	12	3
Region	Karaman	18	2	-	-	6	1	5	1	4	1
	Konya	-	-	-	-	-	-	275	3	34	3
Total Production Area / Yield		**18**	**2**	-	-	**21**	**6**	**320**	**14**	**62**	**9**
5-Year Total Production Area: 421 da											
5-Year Total Yield: 31 tons											
The Black Sea	Düzce	-	-	-	-	-	-	16	1	14	2
Region	Samsun	141	36	144	37	145	36	145	36	130	34
Total Production Area / Yield		**141**	**36**	**144**	**37**	**145**	**36**	**161**	**37**	**144**	**36**
5-Year Total Production Area: 735 da											
5-Year Total Yield: 182 tons											
The Marmara	Balıkesir	4	2	5	1	6	1	6	1	7	1
Region	Bursa	-	-	1	0	1	0	19	4	21	6
Total Production Area / Yield		**4**	**2**	**6**	**1**	**7**	**1**	**25**	**5**	**28**	**7**
5-Year Total Production Area: 70 da											
5-Year Total Yield: 16 tons											
Total by Years Production Area / Yield		**121.127**	**14.724**	**121.472**	**14.477**	**139.061**	**15.895**	**157.074**	**17.965**	**184.711**	**23.866**

2.8. Red paper

When the regions where red pepper is cultivated are examined according to five-year agricultural data, the Southeastern Anatolia Region is in front of all regions with an area of 488.272 decares and 942.464 tons. In second place is the Mediterranean Region with an area of 73,249 da and 139,512 tons. Table 8 contains information on red

pepper cultivation according to years. Accordingly, 228.531 tons of production was realized in 122.415 da area in 2016, and in 2017, the production area decreased to 101.710 decares and the yield decreased to 179.264 tons. In 2018 and the following years, the cultivation area and yield did not change much. If Table 8 is examined in terms of cultivation area and yield amount by regions, in the years 2016-2020, the Southeastern Anatolia Region ranked first in both data, and the Mediterranean Region ranked second. The provinces with the highest cultivation were Şanlıurfa and Gaziantep.

Table 8. Red paper production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Hatay	3.672	9.984	3.720	3.484	3.200	2.690	1.220	1.234	1.237	2.019
	Kahramanmaraş	13.022	27.283	9.269	17.620	13.050	24.625	11.659	23.513	13.200	27.060
Total Production Area / Yield		**16.694**	**37.267**	**12.989**	**21.104**	**16.250**	**27.315**	**12.879**	**24.747**	**14.437**	**29.079**
5-Year Total Production Area: 73.249 da											
5-Year Total Yield:		**139.512 tons**									
The Aegean Region	Aydın	2.025	3.564	2.000	3.800	1.895	3.601	1.855	3.246	1.610	2.673
	Muğla	432	428	20	20	20	24	20	24	20	30
Total Production Area / Yield		**2.457**	**3.992**	**2.020**	**3.820**	**1.915**	**3.625**	**1.875**	**3.270**	**1.630**	**2.703**
5-Year Total Production Area: 9.897 da											
5-Year Total Yield:		**17.410 tons**									
The Southheastern Anatolia Region	Adıyaman	1.600	2.736	800	1.368	1.000	1.710	600	1.026	660	1.320
	Gaziantep	31.350	40.988	42.400	54.900	43.000	59.100	40.000	60.000	43.000	66.603
	Kilis	15.369	17.245	16.500	29.500	16.500	31.500	16.500	31.650	20.566	43.510
	Şanlıurfa	53.095	121.123	25.101	63.252	39.100	98.250	45.355	113.803	35.776	102.880
Total Production Area / Yield		**101.414**	**182.092**	**84.801**	**149.020**	**99.600**	**190.560**	**102.455**	**206.479**	**100.002**	**214.313**
5-Year Total Production Area: 488.272 da											
5-Year Total Yield:		**942.464 tons**									
The Marmara Region	Bursa	1.850	5.180	1.900	5.320	2.100	5.880	2.200	6.160	3.800	10.640
Total Production Area / Yield		**1.850**	**5.180**	**1.900**	**5.320**	**2.100**	**5.880**	**2.200**	**6.160**	**3.800**	**10.640**
5-Year Total Production Area: 11.850 da											
5-Year Total Yield:		**33.180 tons**									
Total by Years Production Area / Yield		**122.415**	**228.531**	**101.710**	**179.264**	**119.865**	**227.380**	**119.409**	**240.656**	**119.869**	**256.735**

2.9. Cumin

The Central Anatolia region was ahead of other regions in terms of total cultivation area (1.401.405 da) and total yield (94.226 tons) in the five-year period between 2016-2020. In the Aegean Region that follows, 1,384 tons of cumin has been produced on an area of 21,718 decares, and the East Anatolian Region (26 da/0 tons) is in the last row.

Table 9. Cumin production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Antalya	-	-	-	-	-	-	20	2	20	2
	Burdur	35	4	-	-	-	-	-	-	74	4
	Kahramanmaraş	100	3	100	6	-	-	50	3	-	-
Total Production Area / Yield		**135**	**7**	**100**	**6**			**70**	**5**	**94**	**6**
5-Year Total Production Area: 399 da											
5-Year Total Yield: 24 tons											
The East Anatolia Region	Malatya	26	0	-	-	-	-	-	-	-	-
Total Production Area / Yield		**26**	**0**								
5-Year Total Production Area: 26 da											
5-Year Total Yield: 0 tons											
The Aegean Region	Afyonkarahisar	3.093	185	4.328	280	5.848	407	3.068	181	4.061	254
	Denizli	562	31	95	7	250	16	100	6	135	7
	Kütahya	48	3	30	2	40	2	25	2	10	1
	Uşak	25	0	-	-	-	-	-	-	-	-
Total Production Area / Yield		**3.728**	**219**	**4.453**	**289**	**6.138**	**425**	**3.193**	**189**	**4.206**	**262**
5-Year Total Production Area: 21.718 da											
5-Year Total Yield: 1.384 tons											
The Southeastern Anatolia Region	Gaziantep	-	-	-	-	-	-	-	-	200	14
	Şanlıurfa	120	6	4.490	269	1.853	93	611	34	57	3
Total Production Area / Yield		**120**	**6**	**4.490**	**269**	**1.853**	**93**	**611**	**34**	**257**	**17**
5-Year Total Production Area: 7.331 da											
5-Year Total Yield: 419 tons											
The Central Anatolia Region	Ankara	171.720	11.357	140.830	10.002	142.855	9.798	133.473	8.199	94.837	5.843
	Eskişehir	4.613	323	2.876	231	2.578	216	1.780	172	1.218	105
	Karaman	500	20	550	22	550	22	580	23	750	30
	Kayseri	7500	450	15.611	785	42.400	2.108	50.277	2.929	15.376	919
	Kırıkkale	602	37	2.001	140	2.000	130	1.900	124	210	14
	Kırşehir	3.950	367	1.343	114	2.129	177	2.045	167	1.180	75
	Konya	75.502	5.770	94.714	7.290	157.726	10.980	123.392	8.073	90.411	6.359
	Nevşehir	23	2	-	-	45	2	516	26	60	3
	Sivas	210	13	60	5	3.250	228	3.838	290	3.397	284
	Yozgat	-	-	-	-	13	1	14	1	-	-
Total Production Area / Yield		**264.620**	**18.339**	**257.985**	**18.589**	**353.546**	**23.662**	**317.815**	**20.004**	**207.439**	**13.632**
5-Year Total Production Area: 1.401.405 da											
5-Year Total Yield: 94.226 tons											
The Black Sea Region	Çorum	220	15	330	22	224	15	200	13	136	9
Total Production Area / Yield		**220**	**15**	**330**	**22**	**224**	**15**	**200**	**13**	**136**	**9**
5-Year Total Production Area: 1.110 da											
5-Year Total Yield: 74 tons											
Total by Years Production Area / Yield		**268.849**	**18.586**	**267.358**	**19.175**	**361.761**	**24.195**	**321.889**	**20.245**	**212.132**	**13.926**

When Table 9 is examined in terms of cultivation area and yield per years, it is seen that a yield of 18,586 tons was obtained in an area of

268,849 decares in 2016, and in 2020, the production area decreased to 212,132 decares and yield deecreased to 13,926 tons. The highest cultivation area and yield was reached in 2018 (361,761 da/24,195 tons). Cumin cultivation is carried out intensively in the Central Anatolia Region, followed by the Aegean Region. The provinces of Ankara and Konya ranked in the forefront in terms of production area and yield by years.

2.10. Coriander

It is seen that coriander cultivation is carried out only in the Mediterranean and Central Anatolia Regions, and according to the total five-year data, the Mediterranean Region (2,129 decares/172 tons) takes the first place, and the Central Anatolia Region (1,799 da/128 tons) ranks second (Table 10). While coriander cultivation was carried out on an area of 503 decares (42 tons) in 2016, it decreased to 155 decares (12 tons) in 2019, but the production area increased to 2,455 decares (188 tons) in 2020. While the Central Anatolia Region ranks first in terms of both production area and yield until 2020, 168 tons of yield was obtained by cultivating in an area of 2,109 decares in the Mediterranean Region in 2020, and it took precedence over the Central Anatolia Region. Similarly, Konya had the highest cultivation area and yield on the basis of provinces until 2020, in 2020, coriander cultivation was started in Burdur in an area of 2,104 (167 tons) and it was ahead of Konya in both data (Table 10).

Table 10. Coriander production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Antalya	5	1	5	1	5	1	5	1	5	1
	Burdur	-	-	-	-	-	-	-	-	2.104	167
Total Production Area / Yield		**5**	**1**	**5**	**1**	**5**	**1**	**5**	**1**	**2.109**	**168**
5-Year Total Production Area: 2.129 da											
5-Year Total Yield: 172 tons											
The Central Anatolia Region	Ankara	-	-	-	-	-	-	-	-	28	1
	Eskişehir	-	-	-	-	-	-	-	-	25	1
	Konya	498	41	405	28	400	28	150	11	293	18
Total Production Area / Yield		**498**	**41**	**405**	**28**	**400**	**28**	**150**	**11**	**346**	**20**
5-Year Total Production Area: 1.799 da											
5-Year Total Yield: 128 tons											
Total by Years Production Area / Yield		**503**	**42**	**410**	**29**	**405**	**29**	**155**	**12**	**2.455**	**188**

2.11. Lavender

When the total production area and total yield values of the last five years are examined on the basis of regions, it is seen that the Mediterranean Region takes the first place with a yield of 27,763 decares and 3,876 tons. Then, there is the Aegean Region with a production of 2,495 tons in 18,206 da area. The least production data are in the Southeast Region with an area of 75 decares and a yield of 6 tons. In Table 11, as a result of the examination of the data of the last five years on lavender production separately, it is seen that the cultivation area and yield increase every year. Thus, the highest production area (22.188 da) and yield (3.499 tons) were reached in 2020. In terms of production area, the Mediterranean Region took the first place and the Aegean Region took the second place in all of the years 2016-2020. In terms of yield, the situation has not changed. Lavender cultivation started in 2020 in the provinces of Hatay,

Kahramanmaraş, Malatya, Manisa, Diyarbakır, Aksaray, Karaman, Kırıkkale, Çorum, Tokat, Istanbul and Kırklareli. When the sowing-planting area and yield amount are examined by provinces, Isparta was the first and Afyonkarahisar was the second in both data.

Table 11. Lavander production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
	Adana	-	-	8	2	9	2	129	10	133	12
The	Antalya	5	1	12	2	12	2	52	8	352	206
Mediterranean	Burdur	-	-	-	-	1.320	122	1.678	156	2.821	274
Region	Hatay	-	-	-	-	-	-	-	-	7	1
	Isparta	3.473	506	3.565	511	3.623	535	4.568	668	5.596	852
	Kahramanmaraş	-	-	-	-	-	-	-	-	400	6
Total Production Area / Yield		**3.478**	**507**	**3.585**	**515**	**4.964**	**661**	**6.427**	**842**	**9.309**	**1.351**
5-Year Total Production Area: 27.763 da											
5-Year Total Yield: 3.876 tons											
The East Anatolia Region	Malatya	-	-	-	-	-	-	-	-	109	20
Total Production Area / Yield		-	-	-	-	-	-	-	-	**109**	**20**
5-Year Total Production Area: 109 da											
5-Year Total Yield: 20 tons											
	Afyonkarahisar	1.700	204	1.750	210	1.960	235	2.321	279	3.327	845
	Denizli	270	29	820	89	938	102	1.108	120	1.760	191
The Aegean	Kütahya	97	0	88	0	98	3	120	4	670	59
Region	Manisa	-	-	-	-	-	-	-	-	180	27
	Muğla	35	5	135	20	151	10	206	16	225	22
	Uşak	-	-	-	-	-	-	27	0	220	25
Total Production Area / Yield		**2.102**	**238**	**2.793**	**319**	**3.147**	**350**	**3.782**	**419**	**6.382**	**1.169**
5-Year Total Production Area: 18.206 da											
5-Year Total Yield: 2.495 tons											
The Southeastern Anatolia Region	Diyarbakır	-	-	-	-	-	-	-	-	30	1
	Şanlıurfa	-	-	-	-	-	-	45	5	-	-
Total Production Area / Yield		-	-	-	-	-	-	**45**	**5**	**30**	**1**
5-Year Total Production Area: 75 da											
5-Year Total Yield: 6 tons											
	Aksaray	-	-	-	-	-	-	-	-	92	32
	Ankara	-	-	-	-	-	-	200	34	833	169
	Eskişehir	10	0	25	0	53	4	83	11	319	37
The Central	Karaman	-	-	-	-	-	-	-	-	378	126
Anatolia	Kayseri	-	-	11	0	11	0	1	0	1	0
Region	Konya	110	2	150	2	178	4	187	10	449	58
	Kırıkkale	-	-	-	-	-	-	-	-	6	1
	Niğde	-	-	-	-	170	0	160	0	240	38
	Sivas	-	-	-	-	-	-	20	2	101	11
Total Production Area / Yield		**120**	**2**	**186**	**2**	**412**	**8**	**651**	**57**	**2.419**	**472**
5-Year Total Production Area: 3.788 da											
5-Year Total Yield: 541 tons											
	Çorum	-	-	-	-	-	-	-	-	48	10
The Black Sea	Düzce	-	-	-	-	-	-	8	2	14	3
Region	Gümüşhane	-	-	-	-	-	-	50	12	160	38
	Tokat	-	-	-	-	-	-	-	-	111	3
Total Production Area / Yield		-	-	-	-	-	-	**58**	**14**	**333**	**54**
5-Year Total Production Area: 391 da											
5-Year Total Yield: 68 tons											
	Bursa	-	-	27	7	27	7	27	7	251	28
	Çanakkale	-	-	-	-	-	-	480	73	1.269	189
The Marmara	Edirne	-	-	-	-	10	1	37	4	130	13
Region	İstanbul	-	-	-	-	-	-	-	-	484	41
	Kocaeli	-	-	-	-	7	0	14	1	-	-
	Kırklareli	-	-	-	-	-	-	-	-	489	47
	Tekirdağ	-	-	15	2	117	13	382	40	983	114
Total Production Area / Yield		-	-	**42**	**9**	**161**	**21**	**940**	**125**	**3.606**	**432**
5-Year Total Production Area: 4.749 da											
5-Year Total Yield: 587 tons											
Total by Years Production Area / Yield		**5.700**	**747**	**6.606**	**845**	**8.684**	**1.040**	**11.903**	**1.462**	**22.188**	**3.499**

2.12. Blueberry

In Table 12, it is seen that blueberry agriculture is carried out in the Mediterranean, Aegean, Black Sea and Marmara Regions. In terms of the total cultivation area and production amount for five years, the Black Sea Region ranked first with a yield of 1.263 tons in an area of 2.911 decares. In the Marmara Region, which ranks second, 579 tons of yield has been obtained on an area of 1,863 decares. When Table 12 is analyzed according to the production years, it is seen that although the production area decreased a little in 2017 (582 da) compared to 2016 (588 da), the yield showed a constant increase in the following years. The highest production area (2.128 da) and yield (1.287 tons) were reached in 2020. Although blueberry was cultivated intensively in the Black Sea Region until 2020, Marmara Region has been ahead of the Black Sea Region with the increase in the agricultural area in Bursa in 2020 (904 da).

Table 12. Blueberry production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Antalya	-	-	-	-	-	-	-	-	450	673
Total Production Area / Yield		-	-	-	-	-	-	-	-	**450**	**673**
5-Year Total Production Area: 450 da											
5-Year Total Yield: 673 tons											
The Aegean Region	Afyonkarahisar	-	-	-	-	-	-	-	-	119	0
Total Production Area / Yield		-	-	-	-	-	-	-	-	**119**	**0**
5-Year Total Production Area: 119 da											
5-Year Total Yield: 0 tons											
The Black Sea Region	Artvin	20	9	26	13	5	9	5	9	44	22
	Giresun	26	5	23	5	23	10	25	9	24	9
	Ordu	-	-	-	-	-	-	-	-	26	5
	Rize	228	99	219	109	286	133	292	168	311	198
	Trabzon	289	54	289	85	296	115	229	100	225	97
Total Production Area / Yield		**563**	**167**	**557**	**212**	**610**	**267**	**551**	**286**	**630**	**331**
5-Year Total Production Area: 2.911 da											
5-Year Total Yield: 1.263 tons											
The Marmara Region	Bursa	-	-	-	-	355	91	479	139	904	264
	İstanbul	25	18	25	13	25	17	25	18	25	19
Total Production Area / Yield		**25**	**18**	**25**	**13**	**380**	**108**	**504**	**157**	**929**	**283**
5-Year Total Production Area: 1.863 da											
5-Year Total Yield: 579 tons											
Total by Years Production Area / Yield		**588**	**185**	**582**	**225**	**990**	**375**	**1055**	**443**	**2.128**	**1.287**

In the provinces of Antalya and Afyonkarahisar, blueberry agriculture started in 2020, so it has become cultivated in all coastal regions. However, in Afyonkarahisar, a yield in tons in an area of 119 da could not be recorded. Following the Black Sea Region, the Marmara Region took the second place, the Mediterranean Region, and the third. If the table is evaluated in terms of 12 provinces, Rize, Trabzon and Bursa, where blueberry cultivation started in 2018, were the provinces with the highest production area and yield.

2.13. Lemon Balm (*Melissa*)

If Table 13 is examined in terms of total production area and total yield of lemon balm in 2016-2020, it is seen that Central Anatolia Region (660 da/327 tons) ranks first. The least production occurred in the Aegean Region with a yield of 5 tons in an area of 10 decares. When the years of agriculture were evaluated separately, in 2016, cultivation was carried out in the area of 213 da and a yield of 108 tons was obtained. Both data decreased in 2017 (207 da/106 tons), 2018 (172 da/84 tons) and 2019 (209 da/93 tons) following this year. However, in 2020, the production area increased to 284 decares and the yield to 150 tons. Lemon balm cultivation in our country was carried out in the Mediterranean, Aegean, Central Anatolia and Black Sea Regions in 2016-2020. The Central Anatolia Region had the highest production area and yield until 2020, and despite having the highest production area (121 da) in 2020, it fell behind the Mediterranean Region in terms of yield. If Table 13 is examined in terms of provinces, it is seen that Karaman ranked first in terms of both production area and yield in 2016-2020, and lemon balm cultivation started in 2020 in Burdur and Hatay.

Table 13. Lemon Balm (*Melissa*) production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Adana	50	23	50	23	50	23	28	13	28	13
	Antalya	5	4	5	5	5	5	5	5	5	4
	Burdur	-	-	-	-	-	-	-	-	51	35
	Hatay	-	-	-	-	-	-	-	-	33	23
Total Production Area / Yield		**55**	**27**	**55**	**28**	**55**	**28**	**33**	**18**	**117**	**75**
5-Year Total Production Area: 315 da											
5-Year Total Yield: 176 tons											
The Aegean Region	Muğla	2	1	2	1	2	1	2	1	2	1
Total Production Area / Yield		**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**
5-Year Total Production Area: 10 da											
5-Year Total Yield: 5 tons											
The Central Anatolia Region	Ankara	-	-	-	-	-	-	1	1	6	6
	Karaman	151	77	145	74	110	52	132	62	115	55
Total Production Area / Yield		**151**	**77**	**145**	**74**	**110**	**52**	**133**	**63**	**121**	**61**
5-Year Total Production Area: 660 da											
5-Year Total Yield: 327 tons											
The Black Sea Region	Düzce	-	-	-	-	-	-	26	3	27	4
	Samsun	5	3	5	3	5	3	15	8	17	9
Total Production Area / Yield		**5**	**3**	**5**	**3**	**5**	**3**	**41**	**11**	**44**	**13**
5-Year Total Production Area: 100 da											
5-Year Total Yield: 33 tons											
Total by Years Production Area / Yield		**213**	**108**	**207**	**106**	**172**	**84**	**209**	**93**	**284**	**150**

2.14. Mint

Table 14 shows the data of mint cultivation in Turkey in the 2016-2020 year. While the planting area was between 10.000-11.000 da and the yield was between 14.000-15.000 tons in 2016, 2017 and 2018; in 2019 the production area increased to 12.650 decares and the yield increased to 16.011 tons, in 2020, the production area increased to 13.110 decares

and the yield to 23.471 tons. Mint cultivation is observed in all regions of Turkey.

Table 14. Mint production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Mediterranean Region	Adana	1.550	2.190	1.450	1.203	1.180	981	1.022	886	501	476
	Antalya	29	22	74	46	79	50	74	46	66	92
	Burdur	30	42	27	37	27	38	24	35	30	41
	Hatay	376	315	340	282	390	319	400	332	287	236
	Isparta	88	36	76	31	76	33	77	37	74	36
	Kahramanmaraş	155	125	214	172	200	160	210	179	218	190
	Mersin	515	773	265	398	265	397	665	998	756	1.427
	Osmaniye	1	1	1	1	1	1	-	-	-	-
Total Production Area / Yield		**2.744**	**3.504**	**2.447**	**2.170**	**2.218**	**1.979**	**2.472**	**2.513**	**1.932**	**2.498**
5-Year Total Production Area: 11.813 da											
5-Year Total Yield:	**12.664 tons**										
The East Anatolia Region	Elazığ	305	332	355	387	360	392	405	442	410	448
	Erzincan	45	36	40	32	30	25	20	17	19	24
	Erzurum	11	4	10	4	10	4	10	4	12	5
	Hakkari	10	4	10	4	10	4	-	-	-	-
	Malatya	93	144	91	144	87	134	79	125	80	127
	Tunceli	1	0	1	0	1	0	1	0	-	-
	Van	-	-	-	-	-	-	-	-	4	5
Total Production Area / Yield		**465**	**520**	**507**	**571**	**498**	**559**	**515**	**588**	**525**	**609**
5-Year Total Production Area: 2.510 da											
5-Year Total Yield:	**2.847 tons**										
The Aegean Region	Afyonkarahisar	45	16	45	16	45	16	45	16	3	1
	Aydın	15	9	15	11	15	11	15	11	15	18
	Denizli	8	2	8	2	8	2	8	2	10	3
	Izmir	153	179	215	329	206	325	190	285	141	168
	Kütahya	9	5	12	6	5	3	5	3	5	3
	Manisa	72	36	72	36	45	23	47	24	43	22
	Muğla	30	20	25	17	25	17	36	24	33	23
	Uşak	80	60	80	60	60	45	65	49	67	50
Total Production Area / Yield		**412**	**327**	**472**	**477**	**409**	**442**	**411**	**414**	**317**	**288**
5-Year Total Production Area: 2.021 da											
5-Year Total Yield:	**1.948 tons**										
The Southeastern Anatolia Region	Adıyaman	15	9	5	4	3	2	5	4	7	6
	Diyarbakır	104	48	105	48	105	47	111	49	80	35
	Gaziantep	5.200	9.685	5.200	9.695	5.250	9.720	6.850	10.395	8.510	18.226
	Mardin	80	12	80	12	60	9	70	11	70	11
	Siirt	12	9	12	9	12	9	12	9	12	9
	Şanlıurfa	172	68	167	66	177	70	333	143	223	102
Total Production Area / Yield		**5.583**	**9.831**	**5.569**	**9.834**	**5.607**	**9.867**	**7.381**	**10.611**	**8.902**	**18.389**
5-Year Total Production Area: 33.042 da											
5-Year Total Yield:	**58.522 tons**										
The Central Anatolia Region	Aksaray	43	15	45	17	71	27	79	31	63	26
	Ankara	137	43	137	50	437	949	503	956	434	765
	Çankırı	54	65	43	52	20	25	9	11	15	19
	Eskişehir	20	16	57	50	50	45	70	63	100	95
	Karaman	700	668	450	418	150	118	140	111	144	115
	Kayseri	6	3	6	3	6	3	6	4	6	4
	Konya	20	12	20	12	20	12	415	93	26	16
	Kırşehir	77	36	77	36	27	11	16	6	13	5
	Nevşehir	10	5	10	5	10	6	9	5	8	4
	Sivas	15	8	15	8	10	5	15	7	10	5
	Yozgat	75	70	85	80	85	80	86	79	102	94
Total Production Area / Yield		**1.157**	**941**	**945**	**731**	**886**	**1.281**	**1.348**	**1.366**	**921**	**1.148**
5-Year Total Production Area: 5.257 da											
5-Year Total Yield:	**5.467 tons**										
The Black Sea Region	Bartın	71	16	71	17	71	16	71	16	71	16
	Çorum	31	17	47	22	35	19	39	19	40	20
	Giresun	12	2	12	2	12	2	12	2	12	2
	Karabük	36	12	38	12	39	12	44	16	26	25
	Kastamonu	23	26	23	26	23	26	23	27	21	24
	Ordu	2	1	2	1	2	1	2	1	2	1
	Samsun	53	41	48	35	45	32	45	32	44	33
	Tokat	140	199	145	199	120	185	115	183	115	184
	Trabzon	21	12	21	12	21	12	21	13	19	12
	Zonguldak	8	1	8	1	8	1	8	1	9	1
Total Production Area / Yield		**397**	**327**	**415**	**327**	**376**	**306**	**380**	**310**	**369**	**318**
5-Year Total Production Area: 1.927 da											
5-Year Total Yield:	**1.588 tons**										

Tablo 14. Mint production areas and yields in Turkey (continue)

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Marmara Region	Balıkesir	6	3	1	1	1	1	1	1	-	-
	Bursa	-	-	-	-	5	2	5	10	16	24
	Çanakkale	3	4	6	8	7	10	11	16	12	17
	Istanbul	138	84	142	85	114	69	113	177	113	175
	Kırklareli	3	4	3	4	-	-	-	-	-	-
	Tekirdağ	13	5	13	5	13	5	13	5	13	5
Total Production Area / Yield		163	100	165	103	140	87	143	209	154	221
5-Year Total Production Area: 765 da											
5-Year Total Yield: 720 tons											
Total by Years Production Area / Yield		10.921	15.550	10.520	14.213	10.134	14.511	12.650	16.011	13.110	23.471

However, the Southeastern Anatolia Region is ahead of other regions in terms of both production area and amount (33.042 da/58.522 tons) in terms of total data for the last five years and in all years of cultivation. The Mediterranean Region took the second place in both data. If Table 14 is evaluated in terms of production provinces, it is seen that Gaziantep ranks first in terms of cultivation area and yield in all the years examined.

2.15. Heather

Table 15 provides information about heather cultivation in our country. Accordingly, a yield of 1,883 tons was obtained in an area of 13,850 da in 2016, but in the following years, both the production area and the amount of yield decreased, the lowest production area (6,860 da) and yield (1,788 tons) appeared in 2020. Heather plant is cultivated only in the Black Sea and Marmara Regions, the Marmara Region has come to the fore both in the five-year total production data (42.916 da/8.918 tons) and when the years are evaluated separately. Balıkesir and Edirne

provinces in the Marmara Region have been the provinces with the highest cultivation and yield.

Table 15. Heather production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Black Sea Region	Samsun	100	35	100	35	-	-	-	-	-	-
	Tokat	1.000	200	1.100	220	1.200	240	1.200	240	1.100	241
Total Production Area / Yield		**1.100**	**235**	**1.200**	**255**	**1.200**	**240**	**1.200**	**240**	**1.100**	**241**
5-Year Total Production Area: 5.800 da											
5-Year Total Yield:	**1.211 tons**										
The Marmara Region	Balıkesir	5.450	481	3.700	1.033	3.630	1.209	3.700	1.243	3.300	1.088
	Edirne	6.300	953	4.644	701	4.569	687	1.768	273	1.660	243
	Sakarya	1.000	214	795	194	800	188	800	195	800	216
Total Production Area / Yield		**12.750**	**1.648**	**9.139**	**1.928**	**8.999**	**2.084**	**6.268**	**1.711**	**5.760**	**1.547**
5-Year Total Production Area: 42.916 da											
5-Year Total Yield:	**8.918 tons**										
Total by Years Production Area / Yield		**13.850**	**1.883**	**10.339**	**2.183**	**10.199**	**2.324**	**7.468**	**1.951**	**6.860**	**1.788**

2.16. Hop

It is seen in the Table 16 that in 2016-2020, in Turkey, hop cultivation was only carried out in Bilecik. According to the total production data of five years, a yield of 9,124 tons was obtained in an area of 16,630 decares. In terms of cultivation area, 2016 had the highest value (3,415 da) and the following years decreased. However, although production was made in the area at 3,308 da in 2020, the yield increased compared to other years and reached 1,908 tons.

Table 16. Hop production areas and yields in Turkey

		2016		2017		2018		2019		2020	
		da	tons	da	tons	da	tons	da	tons	da	tons
The Marmara Region	Bilecik	3.415	1.846	3.300	1.785	3.300	1.785	3.307	1.800	3.308	1.908
Total Production Area / Yield		3.415	1.846	3.300	1.785	3.300	1.785	3.307	1.800	3.308	1.908
5-Year Total Production Area: 16.630 da											
5-Year Total Yield:		9.124 tons									

CONCLUSION

The data of the last five years on medicinal and aromatic plant agriculture in our country have been examined and the results obtained are summarized as follows.

- When the five-year total production and total yield amounts of the regions are evaluated, it is determined that nigella, lavender and mint cultivation is carried out in all regions; it is detected that nettle production is carried out only in the Mediterranean Region and hops production is only in the Marmara Region. In terms of sowing-planting area, Mediterranean Region in anise, rose, nettle, coriander, lavender cultivation; Aegean Region in sage, poppy, thyme cultivation; Southeastern Anatolia Region in red pepper, mint cultivation; Central Anatolia Region in nigella, cumin, lemon balm cultivation; Black Sea region in blueberry cultivation; and, Marmara Region in hop cultivation took the first place.

In sage cultivation, the sowing-planting area has increased over the years, and it has been determined that it is cultivated in all regions except the Eastern Anatolia Region. Aegean and Mediterranean Regions, Denizli and Antalya provinces stand out in terms of production area and yield.

- The highest production area and yield in anise cultivation was reached in 2019, but in general, both data increased over the years. The highest values were obtained in the Mediterranean, Central Anatolia and Aegean Regions; and, Burdur, Konya and Denizli were the provinces with the highest anise cultivation.

- There has been an increase in the area of nigella cultivation in general, but the highest data occured in 2019. The regions where the most nigella farming is carried out are the Mediterranean Region, Aegean Region and Central Anatolia Region, and the provinces are Konya, Burdur, Uşak.

- Rose planting areas have increased over the years, and the highest data have been obtained in the Mediterranean Region and Isparta.

- The most cultivation area and yield in poppy production for capsule procurement was reached in 2019, but both values increased in general. Aegean Region and Afyonkarahisar are in the first place in poppy cultivation.

- Nettle agriculture was not sustainable, it was not cultivated in Antalya in 2019 and 2020, and in Burdur, it started to be cultivated in 2020, but the yield was not recorded.

- Thyme production area and yield increased every year, Aegean Region and Denizli had the highest values.

- There has not been a significant change in the cultivation area and yield after 2018 in red pepper production, and the Southeastern Anatolia and Şanlıurfa and Gaziantep provinces have come to the fore.

- There have been fluctuating values over the years in cumin production, and there has been a decrease in area and yield in 2020 compared to other years examined. When cumin cultivation is evaluated according to regions, Central Anatolia Region, when evaluated according to provinces, Ankara and Konya provinces took the first place.

In coriander cultivation, the production area and yield have decreased every year until 2020, but with the start of production in Burdur in 2020, a high increase has occurred in both data. When the production data were examined, it was seen that cultivation was carried out only in the Mediterranean Region and Central Anatolia Region; and, until 2020, Central Anatolia Region and Konya, in 2020, the Mediterranean Region and Burdur had the highest values.

- When the data of the last five years on lavender production are examined, it has been determined that the production area and yield have increased every year, and the Mediterranean Region and Isparta have come to the fore in cultivation.

The production area and the amount of production have increased every year in the cultivation of blueberry. The Black Sea Region, Rize and Trabzon provinces had the highest production area and yield until 2020, but the Marmara Region and Bursa province were in the first place in 2020.

In the production of lemon balm (Melissa), fluctuating values have emerged over the years and the highest production area and yield was reached in 2020. When the production data are examined by regions, it is seen that Central Anatolia and Mediterranean Regions stand out, and when the provinces are examined, it is seen that the province of Karaman stands out.

- When the production area and yield amount in mint farming are evaluated by years, the year 2020 has the highest values. Although cultivated in all regions of Turkey, Southeastern Anatolia Region and Gaziantep has had the highest values.

- There has been a decrease in terms of the area and yield of the heather cultivation over the years, when the production data are analyzed by regions, the Marmara Region, when examined by provinces, Balıkesir and Edirne provinces took the first place.

- Hop cultivation has presented a fluctuating picture over the years. In our country, cultivation is made only in the Bilecik province of the Marmara Region.

In this study, the production areas and yields of cultivated medicinal and aromatic plants were examined. As it is known, there are many more plants in this class. Other plants of economic importance should be cultivated by paying attention to the factors such as climate, soil and topography of our country.

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CHAPTER 2

THE EFFECT OF STRESS ON THE PRODUCTION OF SECONDARY METABOLITES IN MEDICINAL PLANTS: A REVIEW

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INTRODUCTION

Abiotic stresses affect the growth, development and productivity of plants, especially medicinal plants, and also cause the plant to undergo various morphological reactions such as leaf area decline, premature aging, physiological and metabolic processes such as stomatal closure, and reduction in growth rate, accumulation of antioxidants and solutes, and activity of specific genes (Hughes et al., 1989; Sabagh et al., 2021). Plants' response to abiotic stresses depends on the type, intensity and duration of stress, the stage of stress occurrence, as well as plant species, age and developmental stage of the plant (Pagter et al., 2005). In response to stress, specific genes are expressed and enzymes are produced to trigger certain metabolic pathways that ultimately increase the concentration of soluble substances such as proline, sugar, glycine betaine in cells, and the conditions for water moves into the cells, resulting in increased turgor pressure. Plant cells also have antioxidant mechanisms that protect against oxidative damage (Lima et al., 2002). Protection against photo-oxidation by removing excess energy by non-enzymatic defense systems such as carotenoids, ascorbic acid, anthocyanin, glutathione, tocopherol or by increasing the degradation of reactive oxygen species by enzymatic defense systems of antioxidant enzymes such as superoxide dismutase, catalase, peroxidase, glutathione peroxidase, ascorbate peroxidase (Al-Aghabary et al., 2004). In this chapter, some abiotic stresses are investigated on the accumulation of secondary metabolites in medicinal plants.

1. Drought stress

As global warming progresses, the highest temperature will rise at 5 °C rate per year at the end of the 21st century (Sherwood et al., 2013). Drought will cause drought in many regions of the world and this are likely to become more frequent and extreme drought (Okunlola et al., 2017). one of the most critical environmental stresses is drought stress that lead to changes in processes of growth and development including the activity of enzyme, respiration rate, etc (Okunlola et al., 2017). Drought stress is a non- living stress cause's photo inhibition and temperature stress, which apply great effects on the growth and development of plants. Water deficit is the main reason of drought stress when water potential is sufficiently negative and in most cases this situation was followed by high temperatures and solar radiation (Yuan et al., 2018).

Drought stress is the most acute abiotic stresses causes striking modifications in most plants metabolic activities, including photosynthesis, respiration rate, transpiration, hormonal interaction in metabolism, and enzyme function (Okunlola et al., 2017). For example, growth, leaf water potential and stomatal conductance reduction, and enhancing the deyhdrin gene expression will be induced by moderate drought and intense drought not only can it decrease net photosynthesis, reduction of crop yield and transpiration rate but also in some cases, even it can lead to and plant death (Deeba et al., 2012; Caser et al., 2019; Zhang et al., 2018).

Medicinal plants responses to the drought stress by the effective compounds production with shrinking of soil moisture content, height of plant, leaf number and area, decreased in all phases of growth and stem diameter increased at all stages, in particular in terrible stress and root length increased during flowering stage. Moreover, in reproductive stages, drought stress caused a meaningful decreasing in the flower numbers of than the control condition. The highest essential oil percent was accompanied to mild drought stress at stage of flowering and the maximum content of linalool was due to moderate drought stress in the phase of fruiting (Ghaemi et al., 2019).

Furthermore, the drought can meliorate the production of secondary metabolites (SMs) and practices related to water management in some plants such as S. *dolomitica*. Indeed, drought induced a rise in production of sesquiterpene which is in the terpenoids class that has vital role in the food production, cosmetics and pharmaceutical factories are used as flavors and fragrances in those. Indeed, controlling the drought stress can improve the SMs production in some plants (Caser et al., 2019). In other words, drought stress can cause changes in plants metabolic activities, which include the detention of photosynthesis and cell growth associated with an elevated respiratory rate (Mashilo et al., 2017). In fact, plants are able to active the numerous adoption mechanisms occurring in their undulating growth situations to enhance the functional flexibility under effects of abiotic stress factors without impact on plant key activities (Yang et al., 2018, Arnold et al., 2019) by producing the numerous SMs that play various roles in

reaction to altering the environmental situation, growth and development (Kroymann, 2011, Berini et al., 2018).

The SMs are produced sometimes in the living cells of plant that have insignificant role in the plants primary life that produce them at low concentration proportionate with a plant species growth physiology (Ncube & Van Staden, 2015). the SMs production in plants is related to an adaptive capacity to adopt with stress conditions arising due to the changes in surrounding environment that may effect on complex chemical types production and through signaling pathways and processes will response to the structural and functional stabilization (Edreve et al., 2008).

Many of recent studies shown that different groups of SMs are found in drought-stressed plants including complex phenols, terpenes and alkaloids during in growth by ionic or osmotic stress induction (Niinemets, 2015; Afzal et al., 2017; Piasecka et al., 2017). For instance, concentration and the content of phenolic compounds in *Hypericum brasilience* were seriously increased in plants were cultivated under water-limiting condition by compare to the control (Nogues et al., 1998; Abreu & Mazzafera, 2005). Also, phenolic acids and flavonoids as phenolic compounds have been obtained to be the most wide-spreading groups of plant SMs which produced from the shikimate phenylpropanoid biosynthetic pathway (Quan et al., 2016; Nakabayashi et al., 2014). Accumulation of terpenes in Salvia officinalis was closely associated with higher biomass loss (Nowak et al., 2010).

It seems that the drought stress responses are complex mechanisms. It is obvious that firstly plant recognize stress condition and consequently abscisic acid are accumulated in leaves to reduce transpiration water loss by closing stomata. However, there are detailed metabolism involved in defense response system. Plant SMs play a variety of stress response functions in plant cells (Rejeb et al., 2014; Moore et al., 2014; Gobbo et al., 2017).

Drought promoted the SMs such as flavonoids biosynthesis by oxidative stress (Nakabayashi et al., 2014). The various strategies used by plants to avoid the drought stress-induced oxidative detriment including overproduction of antioxidant metabolites which leads to the inhibition of the oxidative chain reaction (Caliskan et al., 2017). In fact, statistical analysis demonstrated that the antioxidant enzymes activities were closely associated with the SMs production. Among the compounds of SMs, phenolic compounds play vital role in stress tolerance of plants as a natural antioxidant compounds (Quan et al., 2016). The reactive oxygen species (ROS) formation such as H_2O_2 is the primary responses of plants to drought stress (Bhargava & Sawant, 2013; Kocsy et al., 2013). In fact, H2O2 signaling in plants is essential factor for response to stress, defense against stress (Deeba et al., 2012; Koffler et al., 2014). As long-term droughts occur, the selective permeability of membrane the membrane is destroyed. During this process, Lipid peroxidation is produced as a result of reactions with oxidative degradation of lipids such as Malondialdehyde (MDA) which is as a drought stresses indicator (Cheng et al., 2018). Under stress

conditions, ROS-induced lipid peroxidation must be mitigated in plants through activities of biochemical and physiological processes as soon as possible (Cao et al., 2014)

Drought promoted the flavonoids biosynthesis by oxidative stress (Nakabayashi et al., 2014). In fact, drought-induced stress increased SMs production in plants such as the willow plants leaves (Larsomn, 1988) while, under water deficit stress, saponins production was reduced in *Chenopodium quinoa* (Soliz-Guerrero et al., 2002). A study showed that among SMs, the TPC (total phenols) and flavonoids (FC) content increased significantly in response to drought (Hodaei et al., 2018). In similar studies, the role of SMs on quantity of spice and medicinal plants was obviously determined and explained.

Under limited water apply, the content of some compounds, such as isoprenoids, phenols, or alkaloids increased that affect the quality of plants significantly (Kleinwachter & Selmar, 2015). Also, in a case study, the plant biomass production and content of terpenes in thyme plant under drought stress was considered, results showed that the terpenes concentrations (mg/g d.w.) were enhanced in the drought stressed plants in comparison with the control treatment with well-watered condition which associated with higher biomass loss (Kleinwachter & Selmar, 2015). However, increasing in the terpenes accumulation could be originated by two different reasons. Firstly, it could be caused by changes in the reference values: decreasing in growth lead to a lower biomass in stressed plants. Consequently, the natural product biosynthesis rate remains constant – this reason results

in increasing the concentration in the stressed plants. Secondly, all metabolic processes are pushed toward the SMs production.

It seems to be a new insight that the exposure of plants to drought stress, can enhance the SMs production to modify the commodity qualities derived from plants with spice and medicinal properties because it is accepted that these plants cultivated under drought conditions mostly contain elevated SM concentrations than plants cultivated in areas without drought stress.

2. Heat stress

Strong heat waves caused by Global warming which has seriously impact on the plants growth and development. It is accepted that the modification in distinguished pathways of metabolic have prevented the plants adaptive responses to various abiotic stresses. Extreme changes during summers in temperature have a serious effects on agricultural production, since the heat causes the crop yield losses that affect the security of global food in the future. However, the plants have produced the particular adjustments to deal with the detrimental environmental conditions which includes the compatible solutes production that cell turgor can be maintained by stabilizing the osmotic regulation. Even at the molecular level, plants from heat stress can be maintained by various alternation in the genes expression (Shabir et al., 2017). Increasing the content of SMs has been a subject for several recent studies due to their economic values. Therefore, in this study we have

reviewed some of the major effects of environmental stresses such as heat stress on SMs in medicinal plants.

Heat stress is often defined as temperature rising in further the threshold level for a time period of sufficient to cause changeless damage to plant (Essmine, et al. 2010) and heat stress is becoming one of the major abiotic stresses that adversely effects on the plant's growth and development, in particular when predicted increases in earth's global average temperature from 1.4-5.8 degrees Fahrenheit (1 to 3 degrees Celsius) by 2100 based on global climate models (Tacarindua, et al. 2013). In fact, the higher temperature causes forward reactions to occur in plant, including phenological, physiological and molecular responses. Heat stress disrupts the germination, vegetative growth, tiller production, dry matter allocation, reproductive section development, reproductive phases (Boyer and Westgate, 2004; Prasad et al., 2011). In wheat, 10 or 15°C above the optimum of temperature reduce the seeding establishment (Tacarindua, et al. 2013).

The effects of raised temperature on crop yields are investigated in several studies. Heat stress can avoid photosynthesizes which inhibit the seed filling stage that is essential to determine the average seed weight, seed composition and, consequently, qualitative and quantitative yield (Prasad et al., 2017; Sehgal et al., 2017).

Plants have adaptive strategies to cope with abiotic stresses which can survive under changeable environmental conditions at the morphological, physiological, and biochemical levels (Huber &

Bauerle, 2016). Plants indicate the responses in levels of molecular and biochemical after taking signals from surrounding environment such as high temperatures and tolerate the undesirable conditions (Shafiei & Sasine, 2020). Some factors which includes signaling, transcription factors, hormones, and SMs are response to stress, phenylpropanoids and their derivatives are plant SMs. Such compounds including coumarins, lignin building blocks, flavonoids, anthocyanins, and tannins are essential for the function of cell and plant survival to unsuitable environmental conditions, (Fraser & Chapple, 2011). These compounds usually are semi-polar compounds and have a wide range of physiological roles including scavenging the ROS, activation of enzymes, photo protection and regulation the signals (Dixon & Paiva, 1995; Arbona et al., 2013). Furthermore, limonoids have antioxidant activity role in Rutaceae and Meliaceae families. These compounds are naturally-originated SMs derived from isoprenoids (Yu et al., 2005).

In fact, SMs protects plant against abiotic stresses (Hartman, 2004; Kim et al., 2010). Evidences obtained from a large number of research studies have revealed that the plants are coping the oxidative stress by antioxidant and anti-radical functions of the SMs protects (Kim et al., 2010; Selmar & Skleinwachter, 2013). Lipoic and ascorbic acid, o-dihydroxy group-containing flavonoids such as carotenoids, arylamines, quercetin, aliphatic and unsaturated fatty acids among others are the SMs involve chemitypes (Edreva et al., 2008). Under stress, the reorientation of the carbon metabolism changes toward the production of plant secondary compounds (Bryant et

al.,1983) as stress is well recognized as a limiting factor (Edreva et al., 2008). Lily plants have at high level (temperature between 37 and 42 °C). Antioxidant enzymes activities and glutathione contents the improved the heat stress tolerance in lily plants (Yin et al., 2008).

Increasing the ROS production, including hydroxyl radical (●OH), superoxide radical (O2 ●-), singlet oxygen (1O2) and hydrogen peroxide (H_2O_2) are one of the mechanisms of heat stress injury (Yin et al., 2008; Harsh et al., 2016). Lipids, proteins and nucleic acids in membrane will be peroxided when ROS are accumulated that result the disrupt homeostasis. Although, plants show one series of special mechanisms to diminish and repair the consequent ROS damage. These defensive mechanisms enclose the enzymatic systems, including catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPOX), ascorbate peroxidase (APX), glutathione reductase (GR) and peroxidase (POX), and anthocyanins, carotenoids, flavonoids and ascorbic acid as non-enzymatic antioxidants. Also, peroxidases can remove the reactive oxygen species (Yin et al., 2008; Harsh et al., 2016).

The soluble phenolic metabolisms are controlled by different enzymes. Deamination of L-phenylalanine is the first stage to phenylpropanoid skeleton synthesis in plants (Nag & Kumaria, 2018). Phenylalanine ammonia lyase will be catalyzed the reaction as the main enzyme for the phenolic compounds synthesis. Many factors can be affected on the phenylalanine ammonia lyase activity such as biotic or abiotic stress including light, temperature, plant hormones, RNA and protein

biosynthesis inhibitors, drought stress and mineral nutrition. It is announced that phenylalanine ammonia lyase activity will be increased at low and high temperatures due to produce the soluble phenolics (Moura et al., 2017). The transformation of L-Phenyalanine into *trans*-cinnamic acid can be stimulated by this phenylalanine ammonia lyase activity (by deamination reaction), which is the major go-between in the phenolics synthesis (Rivero et al., 2001).

Phenolic compound such as tocopherols, carotenoids, phenolic acids (benzoic acid derivatives and cinnamon acids), flavonoids, and dipropenes are the mainly antioxidant compounds in plants. Phenolic compounds as the plant SMs have a strong potential to scavenge the free radicals. These compounds exist in the leaves, fruits, seeds, roots, and skin of the plants (Zargoosh et al, 2019; Mathew & Abraham, 2006).

Powerful antioxidants which have the lower toxicity and the higher efficacy are an unavoidable necessity. A growing body of research applied technologies to determining and understanding genes closely associated with pathways involved in the PSMs biosynthesis in medicinal plants (Rejeb et al., 2014; Rai et al., 2017). It is highly desired to comprehend and understand their biosynthesis and regulation which could help to develop a genetic intervention strategy for increasing the pharmacologically important metabolites production (Isah, 2019).

3. Cold stress

Temperature is one of the main fastest-changing condition and most important abiotic factors limiting plant growth. Also, approximately 5 percent of the earth's surface is frost free. Occurring the frost have a significant impact on medicinal plants. Most plants are sensitive to freezing during the active growth periods, but freezing tolerance can be developed by plant responses to environmental signals such as exposure to low, non-freezing temperatures and shortening photoperiods in a process termed cold acclimation. Cold acclimation is the changes of anatomy, physiology and metabolism that take place in response to below-optimal temperatures that lessen permanent freeze damage and improve plant fitness (Levitt, 1980). The SMs production such as polyamines, spermidine, spermine and putrescine are produced during physiological processes such as senescence, development and responses to stress (Gill & Tuteja, 2010). Also, SMs in plants are affected by both abiotic and biotic stress. Serious stress in medicinal and aromatic plants can affect the production of SMs. The negative impact of non-living factors on plant such as cold stress leads to the production of ROS in the cellular compartments of plant cell. Here we provide a review of the impact the cold stress on SMs of different medicinal plants. *Zingiber officinale* Roscoe such as ginger, under chilling stress it may characteristically exhibits structural injuries and suffer from metabolic decomposition when they are exposed to chilling stress. Enzymatic activities and photochemical activities were inhibited due to chilling stress and produces ROS species like superoxide,

hydroxyl radicals and hydrogen peroxide leads to cause serious oxidative damage (Li et al., 2014). Production of the polyamines by plants can be attributed to Mechanisms of Environmental Stress Tolerance that could act as elicitors to the production of SMs (Gill & Tuteja, 2010). SMs are a wide range of active compounds for example production of phenolics in plant cell wall as suberin or lignin and the production of chloro-genic acid in (Perez et al., 1997). Occasionally, variations in temperatures may have numerous effects on expression of genes and enzymatic activity of SMs, fluidity, thickness, permeability of membrane in plant cell that can have significant effect on molecules growth and production (Morison & Lawlor, 1999; Shohael et al., 2006).

In most of the higher plants primary metabolite is responsible for the synthesis of SMs and the growing conditions strongly affect the concentrations of various secondary plant products. The significant application of SMs in nutritive, medicinal, food additive, flavor, pharmaceutical and industrially important pharmaceutical. In most of the cases, presence of abiotic stresses the production of SMs is enhances in the aromatic and medicinally important higher plants, which rise up the phytomedicine production and also promote the essential oil production in aromatic plant (Pradhan, et al., 2017).

Ocimum tenuiflorum has been studied for its SMs and genome information. Under abiotic stress such as cold, drought, light and heat stress, it shows different modifications. *O. tenuiflorum* was more defenseless against cold stress among flood and salinity stresses. It directly affects the SMs of the plant under severe treatments of all these

abiotic stresses. It reduces the content of eugenol which is the main SMs of the plant (Rastogi et al, 2019). In another study, Melatonin applied to cucumber (*Cucumis sativus* L.) seeds help to speed up and increase germination under chilling stress (Posmyk et al., 2009). Exposure of cucumber to chilling increased the activities of SOD, APX, glutathione reductase (GR) and GPX, whereas the content of CAT activity decreased (Lee & Lee, 2000). In similar study, the content of peroxidase and APX increased but the production of CAT decreased (Gou et al., 2012). Although the SMs production in plant are under genetic, but abiotic stresses may affect their biosynthesis in plants (Majroomi & Abdollahi, 2018).

4. Light stress

In addition to other stresses, the biosynthesis of SMs can be affected by light that are vital for quality of plant products (Siddiqui & Prasad, 2017). Although, increasing nutritional quality of plant product in particular crops is main objective, not only levels of special SMs but also their crucial activity is an essential factor for medicinal purposes.

Solar radiation includes X-rays, radio emissions, and visible light as well as ultraviolet and infrared radiation. Only a small amount of ultraviolet B reaches on the earth (Caldwell et al., 2003). However, the low acclimation of UV-B in plant can be significantly contributed to expression of UVR8-activated gene. In fact, this gene is associated with the biosynthesis of flavonoids, protection against oxidative stress (Stracke et al., 2010).

In other words, physiological reaction of plants to light condition closely associated with SMs production in growth (Ghosh et al., 2018). Such plant response to light directly can depend on plant species, development phase and Different characteristics of light and light exposure duration (Ghosh et al., 2018; Isah et al., 2018). For example, exposure of plant to high light intensities, blue light and ultraviolet radiations can stimulate production of anthocyanin (Winkel et al., 2001; Radusiene et al., 2012; Miehe et al., 2015; Pedroso et al., 2017; Kawka et al., 2017). Also a study showed that increased light duration induces American ginseng and *C. acuminata* plants to photosynthesis more ginsenoside and CPT respectively, in roots than those exposed to shorter period of light and was confirmed by the expression of genes that participate in its biosynthesis (Li et al., 1996; Hu et al., 2016). In other study, results showed that white light affected the production of taxol and baccatin III in the cell cultures of *Taxus cuspidata* (Fett et al., 1995).

Although UV-B radiation is able to damage DNA or proteins by the generation of reactive oxygen species (ROS) (Coffey et al., 2017), it can use of the adaptation mechanisms against UV-B radiation. One of the adoptive mechanisms against UV-B radiations in plants is the accumulation and photosynthesis of phenolic compounds. In fact, this radiation induce accumulation of SMs such as tannin, salicylate and flavonoids in leaf. Also, the epidermal layer accumulates compounds of phenolic, carotene, xanthophylls, terpenes and flavonoids provides protection against the deleterious effects of UV-B against. Moreover,

when *Catharanthus roseus* exposed to UV-B radiation, biosynthesis and accumulation of vincristine and vinblastine, which are effective anti-lymphoma and leukemia drugs currently in use, is remarkably enhanced (Torres et al. 2016). It is apparent that the influence of light on plant growth and SM is many-sided and relying on the species investigated (Ghosh et al., 2018).

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CHAPTER 3

WEED PROBLEM IN MEDICINAL PLANTS

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INTRODUCTION

Weeds compete for crops and water, minerals, light and light in agricultural production, causing economic losses in quality and yield (Reddy, 2018). In addition, weeds' indirect damage is caused by hosting pathogens and insects, making the harvest difficult and mixing with the harvested product (Capinera, 2005). Weeds are a pest that must be controlled in medicinal plant production, as in other agricultural agricultural production (Hillocks, 1998). Scientific studies on vegetable, fruit and vineyard areas and field crops such as wheat, corn, paddy, potato, cotton, soybean, sunflower, which are widely cultivated, related to the frequency and density of weeds, coverage area, economic damage thresholds, critical period and control methods (Knezevic et al., 2002; Oerke, 2006). It is seen that it was made. However, there are limited studies on weeds and their control, which are a problem in medicinal plant production areas (Hendawy, 2019).

Medicinal plants are plants with sparse production range, but that does not mean that these plants are not important (Chen, 2016). They are produced for vegetative (root, stem, leaf, flower) or generative (seed) different plant parts (Houter & Nederhoff, 2007; Kricsfalusy, 2016). Regardless of the purpose it is produced for, it is necessary to create an environment without weeds in which the development can be healthy during the germination period, active growth period and flowering period (MacLaren et al., 2020). It is important to observe, diagnose and keep records of weeds in medicinal plants production areas, and to determine the appropriate control method (Abouziena & Haggag,

2016). Due to the presence of weeds in medicinal plant production areas, production is limited and input costs increase significantly due to weed control (Carrubba, 2017).

WEEDS IN MEDICINAL PLANTS

For the production of medicinal plants, fields without weeds should be preferred (Dajic-Stevanovic & Pljevljakusic, 2015). It is necessary to prevent the contamination of weeds in these clean fields (Abouziena & Haggag 2016). However, although we choose a clean field or clash to prevent contamination, weeds in the weed seed bank in the soil are present and contamination (by air, water, fertilizer, and other agricultural products, practices, tools) is inevitable (Maqsood et al., 2020).

Weeds compete with the crop, causing them to be stressed (Bagavathiannan, 2017). Weed stress conditions have a negative effect on most cultivated plants (Patterson, 1995). This is also the case for medicinal purposes, however, stress conditions may increase the properties of the active substance on the secondary metabolites secreted by the medicinal plant (Isah, 2019). Therefore, there is a need for scientific studies on these topics. The interaction of medicinal plants with weeds may vary depending on the crop and the species of weeds, as well as the weed density and duration of action (Khan et al., 2017). Non-living factors (climate and soil), living factors (disease, insect and weeds) and applied agricultural maintenance processes in the places where medicinal plants are grown have an effect on the metabolites of these plants (Liliane & Charles, 2020).

Weeds are generally called undesirable plants in medicinal plant fields (Carrubba, 2017). Important weeds seen in the fields of medicinal plants are given in the table below.

Table 1: Common weeds in medicinal plants (UC IPM, 2021)

Scientific Name	**Common Name**
Amaranthus deflexus	amaranth, low
Amaranthus, blitoides	pigweed, redroot
Amaranthus, retroflexus	pigweed, prostrate
Amsinckia spp.	fiddlenecks
Anthemis cotula	chamomile, mayweed
Avena fatua	oat, wild
Avena sterilis	oat, false
Brassica nigra	mustard, black
Bromus tectorum	brome, downy (cheatgrass)
Capsella bursa-pastoris	shepherd's-purse
Chamomilla suaveolens	pineapple-weed
Chenopodium album	lambsquarters, common
Chenopodium murale	goosefoot, nettleleaf
Conium maculatum	hemlock, poison
Convolvulus arvensis	bindweed, field
Conyza bonariensis	fleabane, hairy
Cynodon dactylon	bermudagrass
Cyperus esculentus	nutsedge, yellow
Dactylis glomerata	orchardgrass
Daucus carota	wild carrot
Descurainia sophia	flixweed (tansy mustard)
Digitaria spp.	crabgrasses
Echinochloa crus-galli	barnyardgrass
Elytrigia repens	quackgrass
Eragrostis cilianensis	stinkgrass (lovegrass)
Erodium spp.	filarees
Helianthus annuus	sunflower, common
Kochia scoparia	kochia
Lactuca serriola	lettuce, prickly

Lamium amplexicaule	henbit
Lepidium campestre	pepperweed, field
Leptochloa fascicularis	sprangletop
Lolium multiflorum	ryegrass, ıtalian
Malva parviflora	mallow, little (cheeseweed)
Panicum capillare	witchgrass
Poa annua	bluegrass, annual
Polygonum arenastrum	knotweed, oval-leaf (common)
Portulaca oleracea	purslane, common
Salsola tragus	thistle, russian
Senecio vulgaris	groundsel, common
Setaria viridis	foxtail, green
Sinapis arvensis	wild mustard
Stellaria media	chickweed
Sisymbrium altissimum	mustard, tumble (jim hill)
Solanum nigrum	nightshade, black
Solanum physalifolium	nightshade, hairy
Solanum sarrachoides	nightshade, hairy
Sonchus oleraceus	sowthistle, annual
Sorghum halepense	johnsongrass
Stellaria media	chickweed, common
Tragopogon porrifolius	salsify, common
Tribulus terrestris	puncturevine
Trifolium spp.	clovers, perennial
Triticum spp.	wheats
Urtica spp.	nettle, burning
Xanthium strumarium	coclebur

When examined according to the classes of weeds found in medicinal plants, there are both grass and broad-leaved weeds, generative (coclebur) and vegetative (johnsongrass) multiplying according to their reproduction, and also one-year (chickweed) according to their life cycle, winter (shepherd's-purse). and pigweed wild carrot and field bindweed weeds. This diversity is due to the diversity of medicinal

plants, different soil and climate structures, and the availability of dry and irrigated farming opportunities (UC IPM, 2021). When weeds are examined as families, it is understood that Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Lamiaceae, Malvaceae, Papaveraceae, Poaceae, Polygonaceae, Portulacaceae, Solanaceae, and Zygophyllaceae are common. Of course, it should not be overlooked that the ability of the medicinal plant to synthesize these metabolites, which it has due to its own biology, belongs to them, and the variety and amount of this depend on the plant's adaptation and competitiveness (Hadacek, 2002).

The purpose of growing medicinal plants is important. So the point here is that for which part of this crop is it grown? Accordingly, weed control planning should be done (Sofowora, 2013). Here, the effect of weeds on the secondary metabolites will come to the fore rather than the effect on the medicinal plant (Attia-Ismail, 2015). The general approach in weed control in medicinal plant areas is to protect the metabolites and increase their amount (Carrubba, 2017).

WEED AND MEDICINAL PLANT COMPETITION

Weed plants compete among themselves and crop plants compete with each other, as well as crops with weeds (Reddy, 2018). When determining the most suitable planting / planting norm between and above rows in medicinal plants, it is not to be ignored that the gaps that will occur can be filled by herbs (Gurib-Fakim, 2006). Care should be taken to have the number of plants in a unit area for maximum crop

yield (Van Alfen, 2014). This increases the development, area coverage and competitiveness of the crop with weeds (Kruepl, 2006). While sparse sowing causes weed infestation, too frequent sowing causes poor plant growth (Liebman et al., 2001). Sometimes the large number of plants may use the plant roots and stems, leaves, leaves and fruits to remain small (Goswami and Ram, 2017).

The proper plant density is satisfactory for the plant to absorb water and mineral matter from the soil, reach sunlight and cover the soil (Spitters, 1989). The basic approach here is to cover the soil by the plant and to meet its optimum requirements (Dabney et al., 2001). Action should be taken according to the condition of green parts, seeds or biomass for the purpose of cultivation (Einhellig, 1995). The high number of plants does not mean that the number and quality of seeds will be high (Betty, 1989). Having more plants in a unit area can reduce the yield of fruit / seed (Onat et al., 2016).

Secondary metabolites in medicinal plants are affected by plant density and weed count (Borges et al., 2017). The purpose of producing medicinal plants should be determined clearly (Sofowora, 2013). Whether we are producing biomass or quality secondary metabolite quantity is important (Bourgaud et al., 2001). Sometimes these issues can be correct and sometimes inversely proportional. The yield and composition of secondary metabolites are important in medicinal plant production (Vanisree et al., 2004). For example, there are determinations that as the yield of thyme increases, its metabolites decrease. A different situation is observed in basil, it was determined

that the metabolite yield increased in parallel with the plant yield (Sifola & Barbieri, 2006). The high rate of essential oil in fennel, which is one of the species where essential oils are obtained from seeds, was obtained from sparsely planted areas (Lopes et al., 2009). Different situations may arise in mixed cultivation related to competition within and between plant species (Craine & Dybzinski, 2013). As a result, inter-row and intra-row of medicinal plants are important in terms of agricultural maintenance operations and especially weed competition (Sedara & Sedara, 2020).

WEED IMPACT ON QUALITY AND YIELD

In order for medicinal plants to develop comfortably and give high yield, a production without herbs is required (Alamgir, 2017). The increase in the number of species and densities of weeds causes a decrease in yield (Cousens, 1985). For example, seeds of medicinal plants (fenugreek, fennel, psyllium, milk thistle, garden cress, black, cumin, isabgol, coriander), biomass (basil, fennel, psyllium, garden cress, cornmint, catnip, st john's wort and coriander) and special parts it has been determined that the yield (flowers of saffron, leaves of rose scented, bulbs of tassel hyacinth, shoots of sage) can decrease in the range of 7-97% due to weeds (Carrubba, 2017). While the yield loss from weeds is 34% in Garden cress (Shehzad et al. 2011), it is more than 90% in coriander and fennel products (Carrubba & Militello 2013). When the losses in biomass and weed yield of medicinal plants are examined, it is about 30% in corn mint (Singh & Saini 2008) and around 80-90% in coriander and fennel products (Carrubba & Militello 2013).

Harvest losses were found to be more than 50% in saffron (Norouzzadeh et al. 2007) and 75% in sage shoots (Satvati Niri et al. 2015).

Yield or yield loss can be calculated by means of crop and weed biomass. Preventing light intake, which is one of the most important damages of weeds to medicinal plants, is to yield and quality. It increases by extinction during the germination and early development period of cultivated plants. This situation grows faster than the cultivated plant of weeds. In fact, weeds often grow much faster than a crop; Initial plant growth was slow in perennial crops such as gentian (Radanović et al. 2014), thyme (Zumelzù et al. 1999) or sage (Karamanos 2000), although this problem has also been reported in annual or biennial plants such as fennel. (Yousefi & Rahimi 2014) or coriander, especially in genotypes that do not form a dense basal rosette (Diederichsen 1996). For this reason, it is important to control weed in the early development period, especially in perennial medicinal plants. Early intention is *Nigella sativa* (Nadeem et al. 2013); *Zingiber officinale* (Kifelew et al. 2015) is around 40 days after sowing / planting in weeds such as *Cuminum cyminum*. It is important to prevent weed in the critical period in medicinal plants to prevent stress (Knezevic et al. 2002). Generally, 10 weeds or 10% coverage is accepted as the economic loss threshold. In some species, 1 weed means 1% yield loss.

It is important to elaborate a little more on the quality-related situation. As medicinal plants are cultivated according to their metabolites, perhaps quality is more important than yield. Weeds can be effective in

the development period of the medicinal plant, in the harvest, and even in post-harvest pollution. The weed invader affects the amount and content of essential oil. The essential oil content was reported to decrease of 20–28.6% in leaves of unweeded rose-scented geranium (Kothari et al. 2002) and coriander (Pouryousef et al. 2015), but oppositely an increase in essential oil was found in basil (Sarrou et al. 2016) such as in fatty oil from Milk thistle (Zheljazkov et al. 2006). Weeds mixed with harvested medicinal plants are mixed with distilled plant extract, which can cause undesirable problems (Rajeswara Rao et al. 2007). Weeds found in medicinal plant production areas reduce both the quality and yield of the crop.

WEED MANAGEMENT IN MEDICINAL PLANTS

It is important to identify weeds that are a problem in the production of medicinal plants in terms of the control method to be determined (Hendawy, 2019). The tactics to be used in weed control should increase the efficiency and quality (Leghari et al., 2015). Many physico-chemical methods such as hand picking, plucking, hand hoeing, soil plowing are widely used (McGiffen et al, 2014). However, in recent years, chemical control has been preferred because of its practicality (Abouziena & Haggag 2016). Due to the high labor costs, my tendency towards tactics where manual picking, plucking or harvesting methods are integrated with mechanization is increasing (Edan et al., 2009).

Chemical control is not a very common method in medicinal plants (Abubakar & Haque, 2020). Below is information on some herbicides used for this purpose. However, the use of these herbicides (glyphosate,

paraquat, bentazone, bromoxynil, clopyralid, metam sodium, bensulade, flumioxazin, pendimethalin, oxyfluorfen, diuron, linuron, clethodim and sethoxydim) should be based on the licensing unit and label information of herbicides in your country (UC IPM, 2021). When the Plant Protection Products (PPPs) is examined, it is seen that Linuron herbicide is licensed for anise and cumin plants against broad-leaved weeds. Also, clethodim is licensed against grass weeds in cumin. However, since the herbicides registered in the PPP database are constantly updated, it is important to scan the licensed herbicides from this database and select herbicides according to the label information for which medicinal plant will be selected (PPP, 2021).

Chemicals have been tested for many medicinal plants, including caraway (*Carum carvi*), clary sage (*Salvia sclarea*), coriander (*Coriandrum sativum*), chamomile (*Matricaria recutita*), mint (*Mentha piperita*, *M. arvensis*, *M. spicata* and others), milk thistle (*Silybum marianum*), Moldavian balm (*Dracocephalum moldavica*), fennel (*Foeniculum vulgare*), sage (*Salvia officinalis*), savory (*Satureja officinalis*), ore- gano (*Origanum vulgare*), thyme (*Thymus vulgare*) and many others (Mitchell & Abernethy 1993; Mitchell et al. 1995; Pank 1992; Singh et al. 2011; Zheljazkovet al. 2006, 2010; Zumelzù et al. 1999). Otherwise, other experiments gave different results, and several modifications of essential oil components were recorded; e.g. in chamomile, chemically treated crop had lower chamazulene content (Singh et al. 2011), whereas plants of Moldavian balm treated with trifluralin showed a higher geraniol content (Janmohammadi et al.

2016). As with some vegetables such as tomatoes (Pala & Karipcin, 2021), it should be known that there is little interest in medicinal plants using herbicides (Chen et al., 2016).

It is known that organic approaches are important in medicinal plant production. In this context, there is a need to research or develop alternative and modern non-chemical methods (Raei & Milani, 2014). Using certified seed, developing tolerant varieties, turning to competitive varieties, planting norm, deep plowing, alternation, cover crops, mulching, solarization, thermal methods, allelopathic materials, robotic tactics, drone technology can be integrated for this purpose (Pala et al., 2017). We can say that there are deficiencies in scientific studies about weed problems in medicinal plants. For this reason, there is a need for research on both the problematic weed species and their fight.

CONCLUSION

The presence of weeds in weed fields in medicinal plant production areas causes stress for weeds. This stress results from the competition between plants and weeds, and the severity of the stress is determined by the type of medicinal plant, the type of weeds and the severity of the infestation. According to this rebetab, there are quality and yield losses in different rates in the medicinal plant. Since medicinal plants are grown for aromatic substances and secondary metabolites, unlike other agricultural products, weeds may cause a decrease or loss of the color, taste, odor, and medicinal properties of these crops. One of the important factors affecting the violence of medicinal plant-weed competition is

the economic loss threshold and critical period. Attention should be paid to the weedy period in the early period. The intensity of the infestation increases as weeds tend to grow rapidly and cover the soil during the planting and early germination period of medicinal plants. Until the competitiveness of the medicinal plant can suppress weeds, weeds should be cleaned in order to have a clean field without weeds. Otherwise, losses caused by weeds can reach 60%.

Preventive measures should be taken for weed control, cultural processes should be done, physico-mechanical tactics should be applied, if necessary, herbicide use should be applied. However, it should not be ignored that herbicides can have a negative effect on the metabolites of medicinal plants. In recent years, the interest in the production of medicinal plants by organic method has been increasing. Therefore, agroecological approaches that integrate good agricultural practices such as mulching, solarization, thermal, digital, allelopathy and biological control gain importance for a sustainable weed control in medicinal plant production. There is a need for more scientific studies on the effects of weeds and their control methods on metabolites of medicinal plants.

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CHAPTER 4

ANALYZES IN MEDICINAL AND AROMATIC PLANTS

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INTRODUCTION

Analyses on plants are of great importance in the quality control of herbal materials. Medicinal plants used for various purposes, especially health and food, must comply with certain quality standards. Organoleptic checks are done first on herbal material. Then, macroscopic and microscopic controls are passed. In addition, it is necessary to make qualitative and quantitative chemical tests with the amount of foreign matter in the sample, the amount of ash, the amount of water, the amount of essential oil, optical values, and the values found should be compared with the standard values. The values obtained by these methods for medicinal and aromatic plants must comply with the quality standard values given in the relevant monographs in the pharmacopoeia and codex. If the values found are not within the limits, any impurity or degradation in the material should be considered.

1. THE IMPORTANCE OF MEDICINAL AND AROMATIC PLANTS

Turkey is one of the leading countries in terms of the diversity of medicinal and aromatic plants thanks to its geographical location, climate and plant diversity, agricultural potential, wide surface area (Özyazıcı, 2019). Medicinal and aromatic plants are plants that have many intended purposes such as food, medicine, cosmetics and spices, and are known to have been used for similar purposes since the beginning of human history. While some of the medicinal and aromatic

plants that are subject to domestic and foreign trade in our country are cultivated, some of them are obtained from nature, as in many parts of the world. Medicinal and Aromatic Plants may be exposed to some unwanted changes and contamination during drying, transportation and storage stages after being collected from nature, they can be affected by climate, soil changes, environmental pollution (heavy metals, radioactive rains, etc.) and mostly microorganisms are contaminated, they can carry insects and their larvae. If it is cultivated, there may be a high amount of herbicide and pesticide residue, among other factors. Dust, soil, insects and rodents and debris can also be contaminated during collection, drying and transportation. For this reason, in order to obtain medicinal and aromatic plants in the desired quality, it should be grown with good agricultural practices or collected from suitable areas. Then, the medicinal and aromatic plants obtained are dried properly without contamination and drug is obtained in the desired quality and the necessary analyses are carried out and made suitable for use. As can be seen, the plants are prepared for use by making the necessary analyses according to the area to be used in medicinal and aromatic plants (Anonymous, 2021a, b, c; Faydaoğlu & Sürücüoğlu, 2011).

2. ANALYZES IN MEDICINAL AND AROMATIC PLANTS

2.1. Sensory Analyses

Organoleptic determinations include examinations made with five sensory organs. In the organoleptic method, the sensory organs and features that are used to recognize the unsplit or powdered drug with the naked eye are determined. Appearance, color, size, fracture surface,

surface properties, texture, odor and taste are the basis of organoleptic analysis. For example,

Odor: After it is noted asno odor, weak, distinct or strong in the sample examined by being crushed between the thumb and index finger, if there is odor, its odor is noted as aromatic, fruity, moldy, etc. In samples such as mint, thyme, clove, special-characteristic menthol, carvacrol and eugenol scents are taken (European Pharmacopoeıa, 2007; Yetim & Kesmen, 2012).

2.2. Macroscopic and Microscopic Analyses

2.2.1.Macroscopic determinations: The state of the plant material in nature, the appearance of the flowers, if any, and the morphological appearance are macroscopic examination. In macroscopic descriptions, the family, genus and species characteristics of the plant are also specified (European Pharmacopoeıa, 2007).

2.2.2.Microscopic determinations: In cases where morphological features are not sufficient to identify a plant or drug, it is necessary to look at its anatomical features. The anatomical structure of plant tissues can only be seen when examined under a microscope. In the microscopic determination method, the tissues of the plant are examined with a microscope (European Pharmacopoeıa).

2.2.3.Plant Identification: Plants are diagnosed using the above two methods (European Pharmacopoeıa, 2007).

2.3. Physical Analyses

Physical analyses include analyses such as thousand grain weight, foreign matter, moisture/dry matter, moisture, coarseness, grinding size, brix, pH, refractive index, sieve analysis, specific gravity and color. Moisture, dry matter, coarseness, specific gravity and color analyses are performed in medicinal plants. Physical analyses are also important as the analysis of herbal materials is performed after the plant is dried (European Pharmacopoeıa, 2007; Yetim, & Kesmen, 2012; Gamlı, 2014).

2.4. Chemical Analyses

Substances found in the parts of plants such as leaves, flowers, roots, stems, fruits and seeds are called primary and secondary metabolites and are examined under these two groups. Substances defined as secondary metabolites are generally less than 5% dry weight in medicinal plants and are classified as terpenes, glycosides, alkaloids, tannins, gums, pigments, flavonoids, essential oils etc. among themselves. Some or all of a medicinal and aromatic plant is important due to the secondary metabolites it carries. Here, the diagnosis and determination of these secondary metabolites are examined under "chemical identification methods" (European Pharmacopoeıa, 2007; Yetim & Kesmen, 2012).

There iscarbohydrate, cyanogenetic glycoside (linamarin in flaxseed), flavone glycosides (rutin in buckwheat, etc.), tropane alkaloids (scopolamine, atropine, hyoscyamine in datura), flavonoid (silymarin

in milk thistle), tannin (epicatechin, gallate, epigallocatechin in tea), fixed oil (olive oil) diagnostic reactions in chemical diagnostic methods (European Pharmacopoeıa, 2007; Yetim & Kesmen, 2012).

2.5. Physicochemical Analyses

Physicochemical tests are of great importance in the quality control of herbal materials. Physicochemical tests include determination of density, specific turning angle and refractive index, viscosity, saponification number in oils, unsaponifiable matter, iodine number, melting point and freezing point onset (European Pharmacopoeıa, 2007).

Density: Density (d) is defined as the ratio of the mass of a substance to its volume. In essential oils, it is found by proportioning the weight of the oil at constant temperature to the weight of water. If the amount of oil is sufficient, a pycnometer is used and if it is not sufficient, a 5 µl capillary tube is used (European Pharmacopoeıa, 2007; Gamlı, 2014).

Specific turning angle: Specific turning angle is defined as the turning angle measured in a 1 dm-long tube of a solution containing 1 g of active substance per milliliter. Each active substance has its own specific angle. The method used to measure the specific turning angle is called polarimetry and the instrument is called polarimeter (European Pharmacopoeıa, 2007; Gamlı, 2014).

2.6. Extraction/Distillation Methods

2.6.1. Definition and features of extraction

The word extraction comes from the Latin word '*Extrahere'* (extraction). It describes the process of pulling a substance in a mixture from one phase to another. In the extraction process, it is important to provide the conditions suitable for the chemical structure and physical properties of the active substance to be extracted and to select the appropriate solvent. Vegetable materials are often extracted after drying. There are many parameters that affect the extraction process. These are temperature, pressure, solvent, particle size, time, mixing speed and mixer type, moisture, surfactant effect, pore property of the material (Yetim & Kesmen, 2012; Baydar, 2016).

2.6.1.1 Extraction types

The extraction process is generally carried out in two ways. Respectively, these are batch and continuous type extractions.

2.6.1.2. Extraction methods

It is possible to separate the extraction methods mainly as mechanical and non-mechanical. Mechanical ones are squeezing, drawing, etc. Non-mechanical methods are extraction with solvents and extraction with liquefied gases. Fixed oil extraction is an extraction method with solvents.

Fixed Oil Extraction: The method is based on the principle of extracting the sample with a solvent (n-hexane or petroleum ether), then weighing the residue after removing the solvent. While calculating the amount of

oil, the moisture content of the sample is taken into account. When necessary, calculations are made on dry matter. Fixed oil extraction is a method mostly used to extract oil from seeds (fenugreek, black cumin, dill, etc.) (Yetim & Kesmen, 2012; Baydar, 2016).

2.6.2. Essential oil distillation

2.6.2.1 Definition and Features of Distillation: Distillation is a widely used method for separating substances in a liquid mixture, based on substance distribution between liquid and vapor. The main purpose of distillation is to separate the volatile components in the mixture from the non-volatile component or from each other according to their volatility. In the distillation method, the basis for separation is the vapor pressure and the solubility of the substance (European Pharmacopoeıa, 2007).

2.6.2.2. Distillation Methods: Distillation methods widely used for essential oil production are as follows;

a. Water distillation

It is a process applied mostly to scented and aromatic herbs. The drug, whose essential oil will be extracted, is cut into small pieces and 100 gr is weighed and put into a balloon. 1000 ml of pure water is added on it. It is heated by the balloon heater so as not to exceed 120 ^{0}C. Water and essential oil vapors condense in the cooler with heating. Oil and water are separated from each other in the graduated pipe. When the oil reaches a constant volume (after about 3 hours), distillation is stopped. The amount of essential oil is read in ml in the graduated part of the

Clevenger apparatus (European Pharmacopoeıa, 2007; Türk Farmakopesi, 2004).

b. Steam distillation

Steam distillation is an alternative method of achieving distillation at temperatures lower than the normal boiling point. It is applicable when the material to be distilled is immiscible (incapable of mixing) and chemically nonreactive with water (Anonymous, 2021d).

2.7. Chromatographic/Spectroscopic Test and Analysis Methods

2.7.1. Definition and classification of chromatography

Chromatography was formed by the combination of the Greek words chroma (color) and graphein (writing), and was first used in 1903 by the Russian botanist Michael Tsvett to separate colored plant pigments.

Chromatography is the general name for the separation, recognition and purification of substances in a mixture in a two-phase system, one of which is stationary and the other is mobile phase.

There are three main elements on the basis of the chromatography technique.

- Stationary phase: This phase always consists of a "solid" or "layer of liquid impregnated on a solid support".
- Mobile phase: This phase always consists of a "liquid" or "gas".
- Type of interaction between substances in stationary phase, mobile phase and their mixture: In chromatography, phenomena such as

"surface attachment or adsorption" and "solubility" constitute the basic types of interaction (Skoog & Nieman, 2008).

2.7.2. Classification of chromatography

1-According to the Application Type

- Planar chromatography

  Paper chromatography

  Thin layer chromatography (TLC)

- Column chromatography

  Gas chromatography (GC)

  High pressure liquid chromatography (HPLC)

  Supercritical fluid chromatography

2-According to Separation Mechanisms

-Adsorption chromatography

-Partition chromatography

-Ion exchange chromatography

-Molecular sieve chromatography (Gel chrom.)

-Affinity chromatography (Chemical crom.)

3-According to Mobile Phase Types

-Liquid Chromatography (LC); Liquid- solid, Liquid- liquid

-Gas Chromatography(GC); Gas-solid, Gas-liquid

-Supercritical Fluid Chromatography (SFC); It is a type of chromatography in which substances at critical temperature and pressure (CO_2) are used (Skoog & Nieman, 2008).

2.7.3. Most used chromatographic methods GC

Gas chromatography is the separation of the compounds that make up a mixture by taking advantage of the differences in physical and chemical properties.

Achieving the measurement in a short time and very sensitively reveals the superiority of the method.

Gas chromatography has been widely accepted in the field of chemistry as a suitable method for the analysis and separation of gases and volatile substances (European Pharmacopoeıa, 2007; Skoog & Nieman, 2008).

There are two phases in gas chromatography;

1) Stationary phase (column) made up of a large surface (porous) material placed in a long tube with a small radius.
2) Mobile phase (this phase is gas) that passes easily through the large surface (porous) material in this stationary phase (European Pharmacopoeıa, 2007; Skoog, F. Holler, J.& Nieman, T.A., 2008).

Gas Chromatography Apparatus

The gas chromatographic system is shown schematically in the figure;

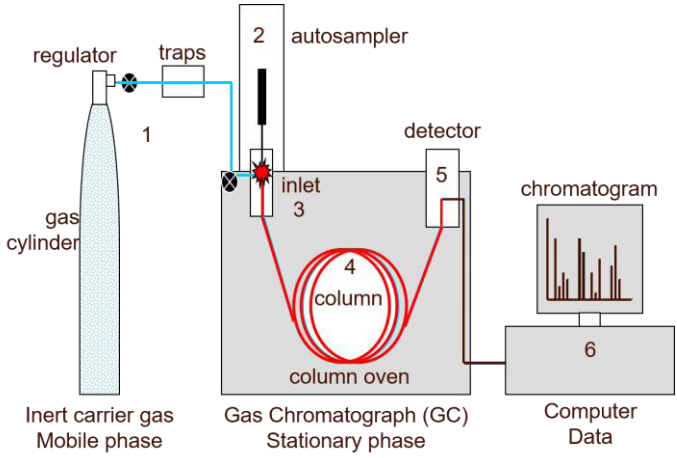

Image 1. Gas chromatographic system

Main Parts of Gas Chromatography Apparatus

1. Carrier gas
2. Autosampler
3. Inlet
4. Analytic Column
5. Detector
6. Pc

GC-MS

GC/MS is a device used for building illumination and quantification by operating GC (Gas Chromatography) and MS (Mass Spectrometer) units together. The device can be used as a GC and GC/MS unit.

Gas chromatography/mass spectrometry is a synergistic combination of two powerful analytical techniques. Gas chromatography separates the components in the mixture. Mass spectroscopy aids in the structural

identification of each component. It has important advantages such as identification of very small samples, strong structural analysis, fast analysis time.

The most popular technique applied to sample a mass spectrometer is gas chromatography. Complex mixtures are first separated by gas chromatography and fed to the mass spectrometer for identification and quantification of each component.

Today, it is used in;

- Biochemistry, biotechnology, petrochemistry, pharmacology,
- Separation of sterols from vegetable oils,
- Genetics, food,
- Forensic medicine toxicology laboratories,
- Separation and analysis in order to determine small amounts of mineral oil and hydrocarbons in Clean water, Waste water, Solid waste and Waste oil samples.

By GC-MS, analyses such as essential oil components (carvacrol in thyme, menthol in mint, linalyl acetate in lavender, etc.), fixed oil components (FAME), residue analysis, pesticides can be performed (European Pharmacopoeıa, 2007; Skoog & Nieman, 2008, Anonymous, 2021e).

HPLC

Liquid chromatography is a separation technique. The components to be separated dissolved in a liquid enter different interactions with the

stationary phase, usually on a solid support, in a column and move at different speeds in the column. They leave the column at different times and thus separate from each other. The liquid, which is the carrier phase, is at a high flow rate since it is pumped to the column with pumps. For this reason, separation takes place in a shorter time and fully. The separated compound is detected with a suitable detector connected to the column outlet and recorded proportionally to its amount. Liquid chromatography systems where separations performed at high speed are made are called High Pressure Liquid Chromatography (HPLC).

High Pressure Liquid Chromatography Apparatus

1. Solvent Resorvoir (Multiple Resorvoir)
2. High Pressure Pump
3. Column
4. Injector System
5. Detector
6. Pc

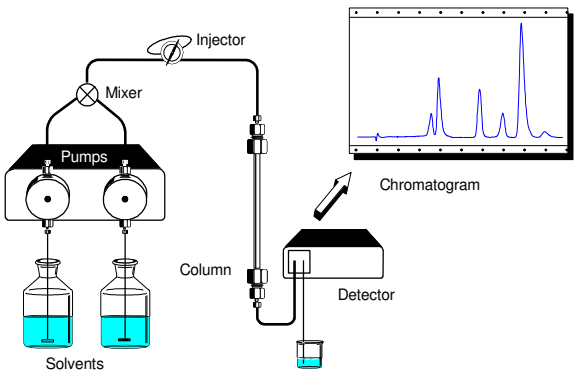

Image 2. HPLC system

Advantages of Hplc

- ✓ HPLC column can be used many times without regeneration.
- ✓ HPLC technique is less dependent on user skill and reproducibility is higher.
- ✓ Quantitative analysis can be used.
- ✓ Analysis time is short.
- ✓ Sensitivity is high.

Analyses in Hplc

Most of the secondary metabolites found in medicinal plants are analyzed by HPLC. Some of these are the silymarin analysis in the milk thistle, the analysis of caftaric acids and alkamides in echniacea, the analysis of trigonellin in fenugreek, the analysis of allicin in the garlic, the routine analysis of the buckwheat, the analysis of the hypericin in the St. John's wort, the analysis of the alkaloid in the poppy, the silymarin analysis in the milk thistle, and the vitamin and amino acid analysis (European Pharmacopoeıa, 2007; Skoog & Nieman, 2008; Baydar, 2016).

2.7.4. Definition and Classification of Spectroscopy

It is the science that studies the interaction between matter and ray.

Spectroscopy is the measurement and interpretation of the electromagnetic radiation absorbed or emitted during the transition of atoms, molecules or ions in a sample from one energy level to another (Skoog & Nieman, 200; Erdik, 1998).

The classification of spectroscopic methods is as follows;

- UV-visible region(VIS) absorption spectroscopy
- Fluorescence and phosphorescence spectroscopy
- Atomic absorption spectroscopy
- Atomic emission and atomic fluorescence spectroscopy
- Infrared spectroscopy(IR)
- Nuclear magnetic resonance spectroscopy(NMR)
- Mass spectrometer

UV-Visible Spectroscopy: The mechanism used to examine the light absorption of the substance is called absorption spectrometer or absorption spectrophotometer. A spectrophotometer assembly consists mainly of light source, wavelength selector (monochromator), detector and the optical signal converted into an electrical signal in the detector is measured with a recorder or a galvanometer (; Skoog & Nieman, 200; Erdik, 1998).

The UV-VIS spectrophotometer is used for the qualitative and quantitative determination of colored inorganic complexes and organic compounds between 600-190 nanometers. It is particularly suitable for the determination of anions that cannot be detected in other devices. US-VIS spectroscopy is often used to measure molecules or inorganic ions and complexes in solution. Many readings are made with the UV-VIS spectrometer. Antioxidant activity determination in medicinal plants, total phenolic substance, total flavonoid determination, hypericin in centaury are some of them (Erdik, 1998).

-*Antioxidant substance:* Antioxidants are defined as compounds that prevent or delay oxidative degradation in foods. These compounds act at the beginning of the oxidative ad autoxidative processes, preventing oxidation and the formation of undesirable reaction products. Broadly speaking, antioxidants can be defined as substances that prevent their negative effects in foods by reacting with oxygen (Skoog & Nieman, 200; Erdik, 1998).

2.8. Pharmacopeia Conformity Tests

Medicinal and aromatic herbs widely used in pharmaceuticals, food and cosmetics are expected to meet certain quality standards. Quality standards for herbal drugs are given in monographs in the pharmacopoeia and codex. Monographs, chemical/ biological/ biotechnological active and auxiliary substances, synthetic and natural compound drugs, finished products or preparate of medicinal products, their definition, content, morphological (appearance), physicochemical (such as solubility, melting and boiling point) and biological (biological activity and definition) are the pharmacopoeia sections that describe their characteristics, identification-diagnostic analysis, packaging, and storage. Studies on the establishment of the Turkish Pharmacopoeia and the European Pharmacopoeia Adaptation are still being carried out. As a result of the tests in the monographs, the values found are expected to be within the defined limits. Organoleptic controls are made primarily in herbal drugs. Then, macroscopic and microscopic controls are passed. After these controls, Determination of Foreign Matter in the Turkish Pharmacopoeia, Determination of All Ash and Ash Insoluble

in HCI, Determination of Moisture (Loss in Drying), Determination of Water by Distillation and Acidity Index in Fixed Oils are analyzed. The necessary analyzes are given according to the active ingredients of the plants given in the pharmacopoeia. As an example, since the plant*Lavandula angustifolia*is an essential oil plant, organoleptic analysis, ash, moisture, thin layer chromatography and analysis of essential oil components are given when looking at the pharmacopoeia. Another example is the determination of silybin and silychristin components found in the seed with the analyses requested in the pharmacopoeia related to the plant*Silybum marianum* (Milk Thistle) and HPLC. Such examples can be multiplied. However, it is not found in many plants used for medicine, cosmetics and food supplements in the pharmacopoeia (European Pharmacopoeıa, 2007; Anonymous, 2021f; Türk Farmakopesi, 2004).

3. CONCLUSION

It is very important to analyze the active ingredients in medicinal plants in accordance with the Pharmacopoeia, to investigate the content of the plant before the analysis and to determine the analysis method according to the active ingredient, to determine the area of use of the material to be used and the methods accordingly. Attention should be paid to issues such as revealing the organic and inorganic compounds in plans used as food raw materials and knowing the content of raw materials used in the pharmaceutical industry and cosmetics. However, it is important to control the conditions in which medicinal and aromatic plants are grown by determining residue, pesticide and toxic

components in plants. Finally, since Medicinal and Aromatic plants are used in many sectors, their monitoring and analysis at every stage from their growing conditions to the final product must be done very carefully and in a controlled manner.

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CHAPTER 5

EVALUATION OF SOME PHARMACOLOGICAL ACTIVITIES OF KENGER (*Gundelia tournefortii* L.)

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INTRODUCTION

The genus *Gundelia tournefortii* L. is a plant belonging to the Astreacea family. In particular, Egypt, Turkey, Iran, Azerbaijan is a naturally growing plant species in temperate regions (Çoruh et al., 2007). Especially plant that grows wild in the Eastern Anatolia region of Turkey is known to grow in different climates and altitudes (Yaldız gülsüm ; çamlıca, 2018). Perennial and single-seeded plant, 20-30 cm long with hairy or glabrous lobes (Sara et al., 2019). The tips of these lobes are hard and barbed (Sara et al., 2019). It is known by names such as 'kenger, tent thorn, mastic grass' in different regions of Anatolia. Kenger is a plant similar to artichoke and its fresh stems are consumed as a vegetable especially in the Eastern Anatolia region. In addition, kenger coffee and gum are obtained from different parts of the plant (Özaltun et al., 2019; Tanker & Tanker, 1967). *G. tournefortii* is used as a medicinal plant in the treatment of various patients in folk medicine. The plant has been used by people in liver diseases (Tabibian et al., 2013), with the belief that it has a hypoglycemic effect, in the treatment of diabetes, migraine (Baydoun et al., 2015) , lung diseases, especially bronchitis, mumps, vitiligo, to prevent inflammation and as a diuretic (Çoruh et al., 2007; Eddouks et al., 2002).

According to the studies investigating the pharmacological effects of Kenger; in the study investigating the liver damage preventive effect of *G. tournefortii* in vivo, it was shown that the plant may have a protective effect depending on the dose. In addition, in vitro cytotoxicity study, it was observed that different concentrations increased cytoprotective

activity in the liver (Jamshidzadeh et al., 2005; Niknahad et al., 2016). In the study investigating the effects of Kenger oil on lipid profiles, it was observed that it reduced the triglyceride level in the liver and plasma atherogenic indices, which are an indicator of cardiovascular diseases (Sharaf & Ali, 2004). It has been shown in different studies that it can be considered as a hypolipidemic agent (Azeez & Kheder, 2012; Hajizadeh-Sharafabad et al., 2016). *G. tournefortii* extract has been reported to reduce inflammation and show analgesic activity in a study conducted on mice (Oryan et al., 2011). It was emphasized that the extracts of Kenger in aqueous, methanol and hexane are effective against HCT-116 human cancer cell line and this activity may be due to phytochemistry in plant content (Abu-Lafi et al., 2019). Besides, antiplatelet (Halabi et al., 2005), antidiabetic (Kadan et al., 2018), anxiolytic (Yuksel et al., 2020) have been reported in different studies to have effects on cardiovascular diseases (Hajizadeh-Sharafabad et al., 2016). The fact that *G. tournefortii* is effective on various diseases shows the medical efficiency of the action mechanisms of the components in the plant. This activity has been associated with flavonoid and polyphenolic compounds, and has been reported to contribute to antiviral, antitumoral, antibacterial and antioxidant activities, according to the studies of the researchers (Apak et al., 2007; Haghi et al., 2011). It has also been reported that phenolic compounds have effects on enzymes that carry out phase reactions in the liver (Çoruh et al., 2007). Today, our eating habits, air pollution, stress, exposure to chemical agents, and our preference for a sedentary lifestyle cause the formation of free radicals in our body. Free radicals weaken

the immune system and cause tissue damage by disrupting the function of cells. One of the important markers of tissue damage is lipid peroxidase. It is known that free fat radicals that develop as a result of oxidative stress caused by lipid peroxidation enzyme inhibition and protein oxidation cause cell death (Alam et al., 2013; Koçak et al., 2020). Antioxidant substances are the components that prevent reactions that will cause many diseases and premature aging, which allow us to be protected from the harmful effects of this balance in the organism. These antioxidant substances are generally obtained by humans from natural origin plants. It is known that flavonoid and phenolic components, vitamins (A, E, C) in the content of plants have antioxidant activities and health benefits (Faydaoğlu & Sürücüoğlu, 2014; Koçak et al., 2020). DPPH (1,1-diphenyl-2-picrilhydrazyl) free radical quenching method is used to evaluate the antioxidant capacity of the extracts obtained from plants by various extraction methods. Since this analysis method is safe and economical, it has been used by many researchers to determine antioxidant activity (Arslan Burnaz et al., 2017; Hara et al., 2018; Jadid et al., 2017; Koçak et al., 2020).

The purpose of this study, *G.tournefortii* plant that grows wild in the mountainous region of Van city in Turkey, DPPH radical scavenging activity, lipid peroxidation and antimirobiyal effectiveness were planned for determine.

1. MATERIAL AND METHOD

1.1. Plant Material

G. tournefortii plants in mountainous area in Turkey's Van was collected in May-June. The collected plant samples were washed first with tap water and then with distilled water. The plant was dried in the shade and ground in an electric mill. It was then placed in an airtight glass jar and stored in a suitable environment for the study.

1.2. Preparation of Plant Extracts

It was stirred at room temperature for 24 hours by maceration method to obtain ethanol (70%) and aqueous extract from the powdered G. tournefortii. Then It was filtered through Whatman Paper No: 1 filter paper. The obtained extract was dried with a rotary evaporator at low pressure and 40°C. The dried plant extract was stored in a properly medium for determination of DPPH radical scavenging and lipid peroxidation inhibitor activities.

1.3. DPPH Radical Scavenging Activity

The DPPH method was modified and its used to measure the radical scavenging activities of the aqueous and ethanol extracts of *G. tournefortii* plant (Blois, 1958). DPPH (0.1 mM) was prepared in methanol and 1mL of this solution was added to 3 ml of the prepared aqueous and ethanol solution at different concentrations (50-500 µg / ml). These solutions were vortexed well, then kept in the dark for 30 minutes at room temperature. Then, absorbance values were read at 517

nm with a spectrophotometer. The radical scavenging activity of DPPH was calculated as a percentage using the formula below (Chen et al., 2020; Koçak et al., 2020; Maduraiveeran et al., 2021)

Inhibition (%) = [(Acontrol – Asample) / Acontrol] x 100

1.4. Lipid peroxidation inhibition activity

The lipid peroxidation prevention activity of G. tournefortii plant was studied by modifying the TBA (Thiobarbituric acid) method (Lo et al., 2005). BHA (Butyl hydroxy anisole) and BHT (Butyl hydroxy toluene) were used as positive controls in this study. BHA was prepared as 30 mg / 10 ml in 97% ethanol solution and likewise in 4 different concentrations of 500, 1000, 1500 and 2000 µg / ml in 70% ethanol solution of the extract. Pre-prepared liver homogenate was mixed with plant extract iron (III) chloride ($FeCl_3$), ethylenediamine tetraacetic acid (EDTA), hydrogen peroxide (H_2O_2) and ascorbic acid, respectively, adding 200 ml of each. Then, It was left to incubate for 1.5 hours at 37ºC. After the incubation, 1200 ml of 28% TCA (Trichloroacetic acid) was added on the mixture and centrifuged at 3000 rpm for 15 minutes. 1200 ml TBA was added on the supernatants obtained and after waiting for 10 minutes at 100ºC, the samples were taken into ice and cooled. Absorbance values were read at 532 nm with a spectrophotometer. The results are plotted according to the following equation, despite increasing extract absorbance values (Koçak et al., 2020; Meydan et al., 2020).

$$\% I = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

1.5. Antimicrobial Activity

In our study, 8 pathogenic microorganisms, *Escherichia coli* ATCC 25952, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATTC 29213, *Candida albicans* ATTC 90028 and *Enterococcus faecium*. Clinical strains of *Klebsiella pneumoniae, Salmonella enterica* were used. Microorganisms were obtained from Van Yüzüncü Yıl University, Department of Molecular Biology and Genetics. The antimicrobial activity of the aqueous and ethanol extract obtained from *G. tournefortii* plant was evaluated using the disk diffusion method (Şapcı & Vural, 2017). Rifampin antibiotic were used for positive control of the study.

2. RESULT and DISCUSSION

In this study, the antioxidant and lipid peroxidation inhibition activity of aqueous and ethanol extracts of *G. tournefortii* plant was determined in vitro.

2.1. Pharmacological Activities

2.1.1. Antioxidant Activity

Antioxidants are defined as compounds that prevent or prevent free radicals from oxidizing by reacting with them. In other words, they are reactions that enable the stopping of free radical-producing reactions and repairing the damage to lipid, protein and DNA molecules. (Karaaslan et al., 2014). Many studies show that foods rich in antioxidants have a protective effect against diseases and their

consumption reduces the risk of heart disease, hypertension, stroke and cancer (Polat & Satıl, 2012).

Various methods are used to determine the antioxidant capacity. One of these methods is DPPH radical scavenging activity. It is one of the frequently used methods to determine the radical scavenging activity of extracts obtained from plants. (Koçak et al., 2020; Maduraiveeran et al., 2021; Onbasli & Yuvali, 2021). According to the DPPH method, the antioxidant activity of the ethanol extraction of the plant was more effective at increasing concentrations than the aqueous extraction. Although the values of both extracts were lower than the positive control groups BHA (95.342-96.442%), BHT (92.108-95.019%), alpha-tocopherol (93.661-95.472%), it was determined that both extracts had antioxidant effects. According to the literature review, it was reported that the methanol extract of the plant showed a significant antioxidant activity when compared with the positive control alpha-tocopherol. (Çoruh et al., 2007). Likewise, karaarslan et al., (2014) showed that *G. tournefortii* plant is a plant rich in antioxidants in their study with three different methods. (Karaaslan et al., 2014). In addition, a different study evaluated that the plant can be used as a source of antioxidants in daily diets. (Konak et al., 2017). The findings of our study are considered to be consistent with the results of previous research and that the plant has antioxidant capacity (Figure 1).

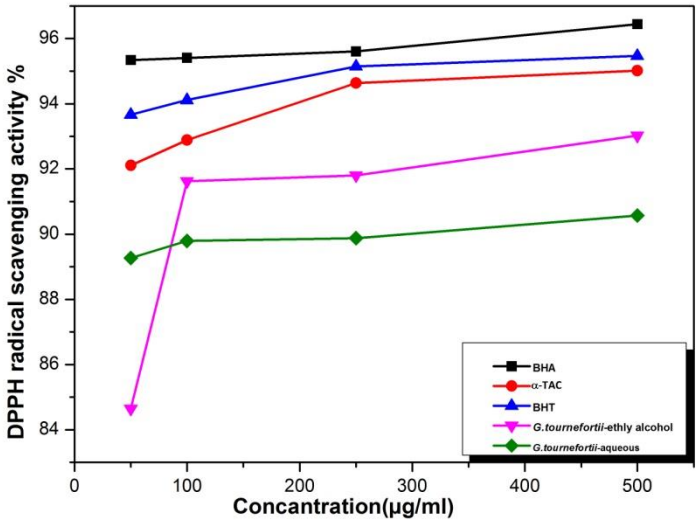

Figure 1. DPPH Radical Scavenging Activity of Different Concentrations of Ethanol and Aqueous Extract of *G. tournefortii* Plant. BHA (Buthly Hydroxy Anisol), BHT (Butyl Hydroxy Toluene), α-TAC (Alpha-tocopherol)

2.1.2. Lipid Peroxidation İnhibition Activity

The degradation of membrane lipids by oxidative damage is commonly referred to as lipid peroxidation. The unsaturated bonds of cholesterol and fatty acids in the cell membrane interact with free radicals to form peroxidation products. In general, aldehydes such as malondialdehyde can also be formed as a result of the breakdown of lipid peroxidation in many biological reactions. When the concentrations of lipid peroxidations increase, the flow rates of the membranes can drop drastically, This may negatively affect enzyme activity. As a result, it

can cause various diseases to occur. (Karaaslan et al., 2014; Meydan et al., 2020; Özdek, 2020).

The lipid peroxidation inhibitory activities of *G. tournefortii* aqueous and ethanol extract were compared to positive controls BHA and BHT. According to the results of the measurements made by spectrophotometer, the lipid peroxidation percentages at increasing concentrations which are positive controls were 93.977-97.089% for BHA and 91.948-95.457% for BHT. Besides, lipid peroxidation percentages of the aqueous and ethanol extracts of the plant in increasing concentrations were found to be 86.146-87.752% and 83.535-93.575%, respectively. According to the results of the study, it is seen in figure 2 that ethanol and water extracts have lipid peroxidation prevention activity in increasing concentrations. In the literature review, it is reported in the study that the methanol extract of *G. tournefortii* has anti-lipid peroxidation activity and has a high phenolic content. (Çoruh et al., 2007). Also, in a different study, it was stated that the plant's MDA (Malondialdehyde) levels were low. In the same study, it was seen that the plant is rich in GSH (Glutathione) and is important for the mechanism of preventing lipid peroxidation due to its low amount of GSSG (Oxidized glutathione). (Karaaslan et al., 2014). In a study on the lipid profiles of the plant, it was stated that it could be good for coronary artery diseases and that the plant was rich in antioxidant properties (Hajizadeh-Sharafabad et al., 2016). The findings of our study are consistent with the literature.

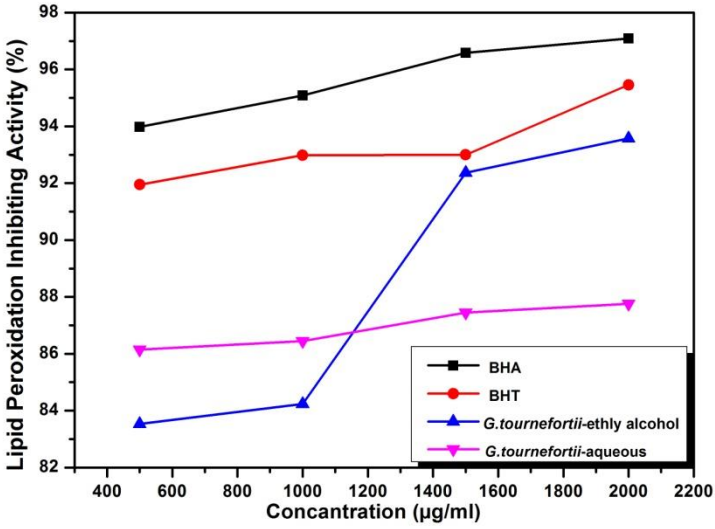

Figure 2. Lipid peroxidation inhibiting activity of *G. tournefortii* plant. BHA (Buthly Hydroxy Anisol), BHT (Butyl Hydroxy Toluene).

2.1.3. Antimicrobial Activity

It was observed that aqueous and ethanol extracts obtained using *G. tournefortii* plant were less effective against some pathogenic bacteria. On the other hand, it was observed that both extracts of the plant formed zones for *E. coli, B. cereus* pathogens and *C. albicans* fungus. It was also observed that ethanol extract formed a zone against *E. faecium, K. pneumoniae* pathogenic bacteria (Table 1). In their study, the researchers reported that the extract obtained from the plant's methanol extract had bactericidal and bacteriostatic effects against certain pathogenic bacteria, especially the root part of the plant, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*,

Staphylococcus epidermis (Darwish & Aburjai, 2010; Haghi et al., 2011; Obeidat, 2011; Samani et al., 2013). Our study is compatible with the studies of other researchers. Both extracts of the plant showed antibacterial effects against *E.coli, B.cereus* pathogenic bacteria.

Table 1: Antimicrobial activity results of *Gundelia tournefortii* extract.

Pathogenic Bacterias	**Aqua**	**Ethanol**	**Rifampin**
Escherichia coli ATCC 25952	8.3	10.5	18.2
Staphylococcus aureus ATTC 29213	-	-	24.2
Enterococcus faecalis ATCC 29212	-	-	-
Bacillus cereus ATCC 10876	8.2	9.4	14.2
Enterococcus faecium	-	10.1	-
Klebsiella pneumoniae	-	10.3	20.1
Salmonella enterica	-	-	-
Fungus			
Candida albicans ATTC 90028	8.4	-	10.5

CONCLUSION

As a result, *G. tournefortii*'s pharmacological effects as a result of researches and its use for various diseases in traditional folk medicine show that it is an important medicinal and aromatic plant. In the study, it was evaluated that both extracts of the plant have antioxidant capacity and prevent lipid peroxidation. it also exhibited antibacterial activity against some pathogenic microorganisms. According to these results, it is necessary to clarify the bioactive components of the plant and determine its pharmacological effects with more detailed studies.

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CHAPTER 6

LIPID PEROXIDATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *Crataegus orientalis* PLANT GROWING IN THE VAN REGION

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INTRODUCTION

Plants are becoming more and more important in the world of Medicine and Pharmacology. Many diseases can be treated with naturally grown plants. Herbal solutions must be supported by scientific research. Some plants may contain a significant proportion of secondary metabolites. Researches to be conducted in this direction may enable the treatment of many pathogen-borne diseases, especially chronic diseases. Van province has an important place in terms of plant diversity. Hawthorn (*Crataegus spp*.), A member of the Rosaceae family, consists of small trees and shrubs that grow in temperate regions (Özcan et al., 2005; Yao et al., 2008). *Crataegus* species are medicinal plants known as flavonoids, vitamin C, glycosy, triterpene acids, proanthocyanidins, saponin, tannin and organic acids used in the treatment of cardiovascular diseases (Ljubuncic et al., 2005; Arslan et al., 2011). Some hawthorn species are used as herbal medicine in many countries such as China, Germany, France and England (Chang et al., 2002). *Crataegus* plant extract can be used as an anti-inflammatory and antioxidant agent in addition to being used in the treatment of cardiovascular diseases (Mills & Bone, 2000). The therapeutic use of extracts obtained from different parts of the hawthorn plant has been around for many years (Bor et al., 2012). Hawthorn plant is a popular herb widely used in traditional medicine to prevent and treat diseases such as angina and hypertension (Edwards et al., 2012). The fruits of hawthorn, which is called "yumuşan" by the local people, have a sour and delicious taste. The

people of the region think that *Crataegus orientalis* (*C. orientalis*) plant is good for cardiovascular health. Considering that *C. orientalis* leaves have antinociceptive and anti-inflammatory effects, the analgesic and anti-inflammatory activities of ethanol extract on mice were investigated (Bor et al., 2012). The antimicrobial activity of hawthorn fruit extract on Micrococcus flavus, *Bacillus subtilis*, *Lysteria monocytogenes* and *Candida albicans* pathogens was investigated (Tadic et al., 2008). Antibacterial activity of medlar and hawthorn extract on *Staphylococcus aureus* and *Klebsiella pneumoniae* microorganisms was investigated (Niu et al., 2013). The consumption of the fruits of this plant as food is thought to have an important place in terms of its use as a landscape plant in terms of the appearance of its flower form and the continuity of wildlife (Bektaş et al., 2017).

In our study, we aimed to investigate the lipid peroxidation, antioxidant and antimicrobial activity of the extract obtained from the *Crataegus orientalis* plant grown in the province of Van.

1. MATERIALS and METHODS

1.1. Preparation of Plant Extract

The leaves and fruits of the *C. orientalis* plant collected from the mountainous areas of Van Gevaş region were brought to the laboratory and washed. Later, the plant parts were left to dry in a place not exposed to sunlight for 15 days (Figure 1) Dried leaves, fruits and seeds were pulverized with the help of a grinder (Meydan & Seçkin, 2021). Ethyl alcohol and water were used to dissolve the powder

extract obtained from the *C. orientalis* plant. The powder extract was taken into flasks after weighing and solvents were added separately. Dissolution was carried out for 36 hours with the help of a magnetic stirrer. Finally, after passing through the evaporator device and removing the appropriate amount of solvents, it was preserved for lipid peroxidation, antioxidant and antimicrobial activity studies (Özdek et al., 2020).

Figure 1. Dried leaves and fruits of the *C. orientalis* plant.

1.2. Lipid Peroxidation İnhibition Activity

The lipid peroxidation inhibitory activity of *C. orientalis* plant extract was found using the thiobarbituric acid (TBA) method (Lo et al. 2005). BHA and BHT were used as positive controls in this experiment. A 10 mg / 10 ml solution of BHA and BHT in 97 % ethanol solution was also prepared from *C. orientalis* plant extract in 4 different concentrations of 500, 1000, 1500 and 2000 µg / ml in 70% ethanol solution. On these prepared solutions, 200 µl of pre-prepared

liver homogenate and 200 µl extract were mixed with 200 µl $FeCl_3$, 200 µl EDTA, 200 µl H_2O_2, 200 µl ascorbic acid and then vortext. It was then left to incubate at 37 ºC for 1.5 hours. After the incubation, 1200 ml of 28 % TCA was added to the mixture. It was centrifuged at 3000 rpm for 15 minutes. After the supernatants were taken, 1200 µl TBA was added and the samples were kept in ice for 10 minutes at 100 ºC and the absorbance values were read at 532 nm in UV.

% Inhibition values against increasing extract concentration were plotted. % inhibition values were calculated according to the equation below.

$$I = [(Akontrol-Asample) / Akontrol] \times 100$$

1.3. Antioxidant Activity

The DPPH extinguhishing activity of *C. orientalis* plant was calculated using the previously found method (Blois, 1958). BHA and BHT were used as positive controls in this procedure. The experiment was performed using 0.1 mg/ml DPPH methanol solutions. DPPH and extracts in the same ratio were prepared in 4 different concentrations of 50, 100, 250 and 500 µg / ml. 3 ml of plant extract and positive control were taken and DPPH solution was added on them. The mixtures formed in the tubes were incubated for 30 minutes at room temperature in the dark. At the end of this period, absorbance values were read at 517 nm.

$$\% I = [(Akontrol-Asample) / Akontrol] \times 100$$

As a result of these processes, a graph of the concentration of *C. orientalis* plant was obtained against the increasing DPPH ethanol concentration (Figure 3). This graph is obtained using the above equation.

1.4. Antimicrobial Activity

The extract obtained from the *C. orientalis* (alıç) plant was applied to eight different pathogens. The test microorganisms used in the study were identified as *Acinetobacter baumannii, Bacillus cereus* ATCC 10876, *Bacillus subtilis, Enterococcus faecium, Klebsiella pneumoniae, Salmonella enterica, Staphylococcus aureus* ATTC 29213, *Candida albicans* ATTC 90028 (Fungus). Disk diffusion method was used for antimicrobial activity (Seçkin & Meydan, 2021). Clinical and reference strains used in the study were obtained from Van Yüzüncü Yıl University Research and Application Hospital. Pathogens were propagated on Müller Hinton Agar medium. In addition, Oleandomycin antibiotic was used as positive control.

2. RESULT and DISCUSSION

2.1. Lipid Peroxidation İnhibition Activity

The degradation of membrane lipids by oxidative damage is commonly referred to as lipid peroxidation. The unsaturated bonds of cholesterol and fatty acids in the cell membrane interact with free radicals to form peroxidation products. It is known that radicals that cause aging of organisms and progression of cancer are involved in lipid peroxidation (Meydan et al., 2020). The lipid peroxidation

inhibition activity of *C. orientalis* plant for ethanol solution was investigated. In this experiment, the lipid peroxidation inhibitory activity of *C. orientalis* ethanol extract was found to be between 67.47 % and 80.17 µg/ml at the lowest and highest concentrations, and these values were between 62.45 % and 74.6 % for the water extract (Figure 2). Lipid peroxidation studies for *C. orientalis* have not been found in the literature. In comparisons with different plants, the activity of *C. orientalis* plant to prevent lipid peroxidation is remarkable (Serçe, 2012; Koçak et al., 2020).

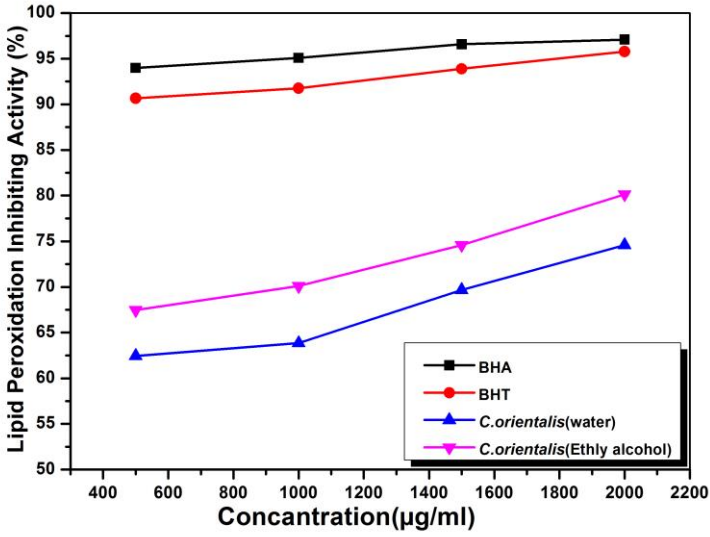

Figure 2. Lipid peroxidation inhibition activity of *C. orientalis* plant in different extract.

2.2. Antioxidant Activity

The ethanol solution of DPPH is a purple-colored nitrogen free radical. DPPH radical is a highly reliable, cheap, accurate, fast, easy and economical method used to evaluate the free radical capture activity of natural antioxidants (Deng et al., 2011). In this experiment, the DPPH radical scavenging activity of *C. orientalis* ethanol extract was between 56.02 % and 72.42 µg / ml at the lowest and highest concentrations, while these values were between 51.65% and 63.69 % for the water extract (Figure 3). The findings obtained in previous studies with *C. orientalis* plant seem to be in line with our current study (Bor et al., 2012). Numerous studies have so far been carried out on the DPPH radical quenching activity of plants. When compared with the studies conducted, it is seen that the radical quenching activity of *C. orientalis* plant is significant (Koçak et al., 2020; Parvu et al., 2014).

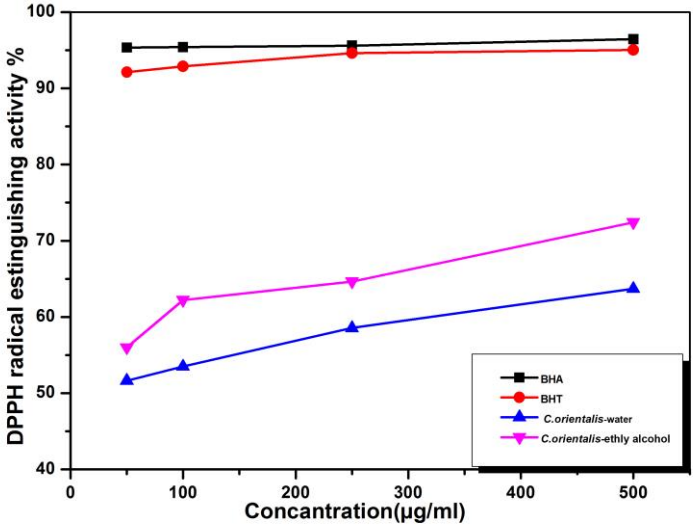

Figure 3. DPPH radical extinguishing activity in different extract of *C. orientalis* plant.

2.3. Antimicrobial Activity

The antimicrobial activities of the aqueous and ethanol-containing extracts obtained from the *C. orientalis* plant were examined using the disk diffusion method (Table1). According to the studies conducted in different species of the genus Crataegus, it was determined that the *Crataegus* tanacetifolia plant extract had an antibacterial effect on *Bacillus subtilis, Shigella, Staphylococcus aureus* and *Listeria monocytogenes pathogens* (Benli et al., 2008). Some parts of the *Crataegus azarolus* plant have been found to be effective against *Staphylococcus aureus* and *Streptococcus faecalis* (Belkhir et al., 2013). In our study, it was observed that zones varying between 8.1-

12.3 were formed against pathogens. While the extracts showed antibacterial effect against the bacteria used, they did not show antifungal effect against *Candida albicans* ATTC 90028. It is thought that solvents such as ethyl alcohol will increase the efficiency of extracts (Çınar et al.,). When looking at the results, it was seen that the extract using ethanol as a solvent was more effective than aqueous extract.

Table 1: Zone diameters (mm) of extracts obtained from *C. orientalis* plant against test microorganisms.

Test Microorganisms	**Ekstrakt (Water)**	**Ekstrakt (Ethanol)**	**Oleandomycin (Antibiotic)**
Acinetobacter baumannii	-	10.1	12
Bacillus cereus ATCC 10876	8.4	10.2	22
Bacillus subtilis	8.1	9.1	22
Enterococcus faecium	9	12.3	11.4
Klebsiella pneumoniae	-	9.5	20
Salmonella enterica	9.1	9	26
Staphylococcus aureus ATTC 29213	9.4	10.2	20
Candida albicans ATTC 90028 (Fungus)	-	-	10.2

CONCLUSION

Despite the advanced developments in the pharmaceutical industry and modern medicine, the need for medicinal plants is increasing. Especially the resistance of pathogen bacteria to existing antibiotics has revealed the necessity of herbal research. In our study, it has been

observed that *C. orientalis* plant has an antibacterial effect. In addition, when the antioxidant analysis values were examined, it was determined that important results emerged. As a result, it is thought that this plant can be used in the production of pioneer in the field of Pharmacology after detailed content analysis.

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CHAPTER 7

EVALUATION OF THE ANTIOXIDANT CAPACITY OF *Salvia virgata* Jacq. GROWN IN SEMI-ARID CONDITIONS

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INTRODUCTION

Medicinal and aromatic plants are commonly used plants as pharmaceutical raw materials in order to protect our current health and cure diseases in traditional and modern medicine, as a nutritional supplement to give taste and aroma to meals, and as herbal tea. In addition, the essential oils they contain are raw materials of the perfume and cosmetic industry (Cheminal et al., 2020; Petrakou et al., 2020).

The first written records on the use of plants in curing diseases date back to 5000 BC, to the Chinese, Indian and Near Eastern civilizations, and it was determined that approximately 250 herbal drogs were used during these periods. Today, the use of herbs for therapeutic purposes varies according to the development level of the countries. While an average of 80% of the population in developing countries uses herbal products for treatment purposes, this rate is less in developed countries (for example, 40-50% in Germany, 42% in the USA, 49% in France) (Budak & Acibuca, 2018).

Turkey located at the intersection of three floristic region (Euro-Siberian, Mediterranean, Iranian-Turan) is very rich in different plant species due to climate and ecological conditions. 9.753 natural species exhibit distribution in the flora of our country. The total number of species and subspecies taxa is 11.707, the number of endemic taxa is 3649 and the endemism rate is 31.82% (Guner et al., 2012).

There are 174 families in the flora of Turkey. One of the richest families in terms of number of species is Lamiacea (Labiatea) family known as Ballıbabagiller. Sage, a member of this family, is the general name of the species included in the genus *Salvia*. Sage got this name from the word "Salveo" which means 'to save' and 'to protect' in Latin (Karabacak, 2009). The genus Salvia, which spreads throughout the world in tropical and subtropical regions and in Central Europe, especially in the Mediterranean region, is represented by approximately 900 species in the world. In our country, as a result of the recent revision studies, it is stated that 99 species of *Salvia* genus, 51 of which are endemic, show natural distribution (Guner et al., 2012).

Taxon within the *Salvia* genus are generally fragrant and herbaceous or bush plants. Although most of them are perennial, there are also biannual or annual types. Sage species exhibit an upright or horizontal development. Stem may be hairy or hairless. Sage leaves are usually long-stemmed and have gland hairs on them. Species have flowers with petals of different colors such as blue, red, white, purple, violet. The essential oil of sage is mostly stored in leaves, flowers in medium-level and least in stems (Grdiša et al., 2015). Sage species have a great importance and a wide market potential in medicinal and aromatic plants due to their biological effects (antioxidant, antifungal, antibacterial, antiseptic, anticancer, etc.) and the oils (essential oils, aromatic oils) they contain. The most commercially valuable are *Salvia officinalis* L. (medicinal sage or dalmatian sage), *S. fruticosa* Mill. (syn.

S. triloba L.) (Greek or Anatolian sage), *S. pomifera* L. (apple sage), *S. lavandulaefolia* Vahl. (Spanish sage) and *S. sclarea* L. (clary sage).

The therapeutic feature of *Salvia* species is due to the essential oils, bioactive components they contain and their high antioxidant activities. In this direction, sage is used for the treatment of many diseases such as colds, throat infections, stomach and abdominal pains, diarrhea, diabetes, high blood pressure, rheumatism, skin diseases (Perry et al., 2003; Walch et al., 2011; Grdiša et al., 2015;).

In this study, it was aimed to investigate the antioxidant capacity of the extracts of *S. virgata* grown in culture conditions using different solvents.

1. THE GENERAL CHARACTERISTICS OF *Salvia virgata* Jacq.

Salvia virgata is a perennial, coarse herbaceous plant that is widely distributed in Southeast Europe and Southwest Asia. It prefers many different habitats such as hard bushes, empty fallow fields, roadsides, forests, meadows, volcanic rocks. This species is naturally growing virtually anywhere in Turkey (Figure 1). This plant, which spreads up to about 2300 m above sea level, is highly resistant to frost (Singhurst et al., 2012; Bayram et al., 2016).

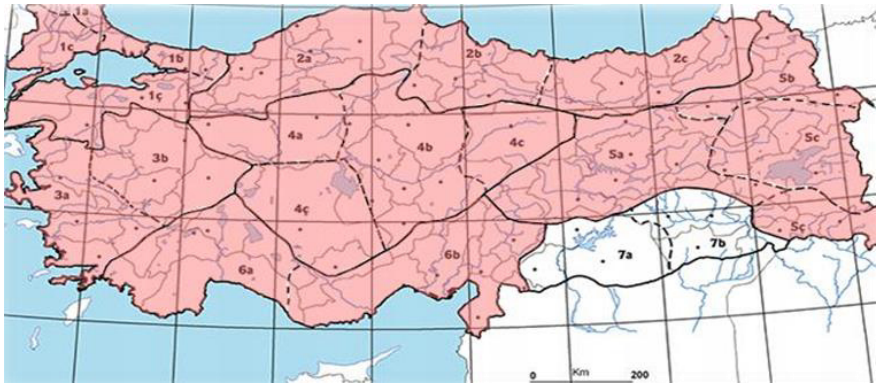

Figure 1: The Natural Distribution Area of *Salvia virgata* in Turkey (Celep & Kahraman, 2012)

It is known by the names of "fatmanotu, yılancık and yağlısomara" among the people. Plant height can vary from 20 cm to 160 cm. The plant stem has an upright and branching structure from above. The leaves are simple, usually lined up on the stem or rarely limited to rosette leaves at the base. Flowering occurs from May to September. The flowers are in the form of compound clusters and the petals have purple, blue, lilac and very rarely white color (Singhurst et al., 2012) (Figure 2). In addition to being a high-value herb used in medical applications, *S. virgata* is used for healing wounds, skin diseases and gynecological diseases among the people (Bayram et al., 2016).

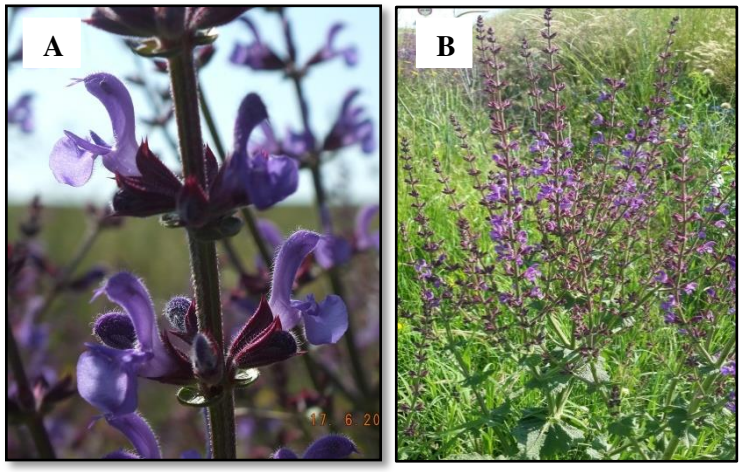

Figure 2: Flowers (A) and aerial parts (B) of *S. virgata.*

2. FREE RADICALS AND ANTIOXIDANTS

Molecules with unpaired electrons in their outer orbits that are occurred during the normal physiological functions of our body are called "free radicals". Free radicals are unstable and highly reactive due to their unpaired electrons. These reactive species cause many diseases such as cancer, cardiovascular diseases, cataracts, weakening of the immune system, premature aging and diabetes by damaging the materials forming the cell structure such as proteins, fats, carbohydrates and nucleotide coenzymes (Halliwell, 2012; Ifeanyl, 2018). Bioactive substances that prevent the formation of free radicals or significantly reduce the negative effects they cause are called "antioxidants". Antioxidants can be produced by body cells and found naturally in foods, or they can be added later in the food industry to preserve the quality and nutritional value of products (especially fats).

Antioxidants can be examined in two classes, natural and synthetic. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), and propyl gallate (PG) are examples of synthetic antioxidants that are currently commercialized (Taghvaei & Jafari, 2015) Vitamins (A, C, E vitamins), carotenoids and phenolic compounds are the most important natural antioxidants that occur naturally in plant and animal tissues or that are released by the processing of food (Lourenço et al., 2019). The most important factor in the antioxidant effect of herbal products is due to flavonoids, cinnamic acid derivatives and phenolic compounds such as coumarins.

3. MATERIAL AND METHOD

3.1. Material

In this research, *S. virgata* seeds collected from natural area were sown on trays containing peat on 12.03.2018. The seedlings that reached sufficient size (approximately 10 cm) in the greenhouse were then planted in the Yozgat Bozok University, Faculty of Agriculture, Topçu Research and Application area. After the seedlings were planted, irrigation was done; no more irrigation was done until harvest. Hoeing has been made for weed control when necessary. Plants were grown in semi-arid conditions. The aerial parts (Flower+Stalk and Leaves) of the plants that have completed their development in a healthy way were collected on 02.10.2018 to be used as trial material and left to be dried in the shade (Figure 3).

Figure 3: Flowers (in the left) and Leaves (in the right) of *S. virgate*

3.2. Soil Characteristics of the Trial Area

Soil analysis results of the trial area are presented in Table 1.

Table 1: Soil Characteristics of the Trial Area

VARIABLE	MEASUREMENT	VALUES
Clay (g/kg)	476	-
Silt (g/kg)	138	-
Sand (g/kg)	386	C
pH	7.09	Neutral
Salt (%)	0,178	Slightly salty
$CaCO_3$ (%)	7.15	Medium calcareous
Organic matter (%)	2.49	Medium
Total N (%)	0.15	Enough
P (µg/g)	78	Excess
K (µg/g)	728	Excess
Ca (µg/g)	7060	Excess
Mg (µg/g)	5604	Overmuch
Fe (µg/g)	8.08	Excess
Cu (µg/g)	2.84	Enough
Zn (µg/g)	0.62	Little
Mn (µg/g)	4.07	Little

Considering the measurement values in Table 1, it is understood that the soil of the trial area contains a medium level of organic matter with 2.49%. There is no deficiency in the amount of total Nitrogen (N) and

available Phosphorus (P), which are of great importance for plant health and development. There are no changeable Potassium (K), Calcium (Ca) and Magnesium (Mg) deficiencies in the table. The soil of the trial area, which is observed to be slightly salty and moderately calcareous, is sufficient in terms of Iron (Fe) and Copper (Cu), which are essential micro nutrients in vulnerable form, but insufficient in terms of Manganese (Mn) and Zinc (Zn). Considering all these results, it is understood that the soil of the trial area has a heavy structure (Yakupoglu, 2018).

3.3. Climatic Characteristics of the Trial Area

Climate characteristics of the experiment area are presented in Table 2.

Table 2: Climate Characteristics of the Experiment Area in 2018 Year

	Total Precipitation (mm)	**Average Temperature (°C)**	**Average Relative Humidity (%)**
January	98.7	0.2	80.4
February	30	4.6	98.3
March	147.2	7.5	67.4
April	20.6	12.2	-1.5
May	114.6	14.8	66.9
June	38.8	24.5	58.2
July	3	21.3	53.2
August	0	20.9	49.4
September	1.9	16.9	55.2
October	43.8	16.1	53
November	34.2	6	71
December	155.3	1.6	81.8
TOTAL	**688.6**	-	-
MEAN		**12.22**	**26.27**

The average monthly total precipitation amount of Yozgat province, where the trial was conducted, between 1929 and 2018 is 562.5 mm,

and the average temperature value is 9.1 ˚C. Considering the average of 1929-2018 in 2018, when *S. virgata* seedlings were planted in the experimental area, it is seen that the average amount of precipitation was 126.1 mm higher and the average temperature 3.12 ° C higher (MGM, 2019).

4. METHOD

4.1. Preparation of Extracts

After the aerial parts of *S. virgata* were harvested, it was separated into flowers and leaves, and dried in the shade. The dried plant organs were ground with the help of a laboratory blender. 4 g of the ground samples were weighed and transferred to 50 ml falcon tubes and 40 (1/10 w / v) ml of methanol was added as solvent. The samples were incubated in the oven (Elekto-mag M 5040 P) for 24 hours at 40 ° C after the solvent addition. The prepared samples were filtered into balloon flasks using Whatman No 1 filter paper, and then methanol was evaporated with the help of a rotary evaporator (Heating Bath B-491, BUCHI). After the removal process was completed, the flasks were kept in the oven for 24 hours to dry completely. Then, 2 ml of methanol was added to the dry plant extracts in a flask, and the extracts used in the study were obtained by passing through the vortex device. The extracts were kept at +4 ° C by closing their mouths with parafilm until analyzes were made.

The amount and yield of the extracts were calculated according to the formula below;

Amount of Extract = Extract + Flask - Remain of dissolve

Efficiency (%) = (V1 × 100) / V2

V1 = weight of extract obtained after drying by removing solvent

V2 = sample weight obtained from *S. virgata* (4 g)

4.2. Determination of DPPH Radical Scavenging Activity

Free radical activities of the extracts were determined by using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical (Gezer et al., 2006). In the first step, the amount of extract that neutralizes a certain amount of DPPH radical was determined. A comparison was made between the samples determined. For the determination of DPPH radical scavenging activity, 16 mg DPPH was dissolved in 100 ml of methanol without any residue and the DPPH solution to be used in the analysis was prepared as 0.1 µl. DPPH reading was made by adjusting 517 nm in the spectrophotometer. Dilution with methanol was continued until the absorbance value was 1.000 ± 5. 1 mg / ml extract solution was prepared as the main stock and 6 different concentrations (50, 100, 150, 250, 500 µg) were formed by dilution. 3 ml of sample was drawn from each concentration and 1 ml 0.1 µl DPPH was added. Ready samples were kept in the dark for 30 minutes. BHT (butyl hydrocytoluene) and BHA (butyl hydroxyanisole) were used as standard antioxidants in the study. Each sample was applied in 4 replications and DPPH radical scavenging activity was determined in% with the formula given below.

% DPPH scavenging activity = [(A control - A extract) / A control] × 100

A control: Absorbance value of the control value containing only DPPH radical solution.

A extract: The absorbance value measured after the addition of the DDPH radical containing solution onto the sample.

Spectrophotometric measurements of DPPH radical scavenging activity were performed with PerkinElmer Lambda 25 UV / VIS spectrophotometer device.

4.3. Determination of Total Phenolic Content (Folin Method)

Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenolic content of the extracts obtained (Singleton et al., 1999). In order to do the study, 100 ml sodium carbonate (Na_2CO_3) solution was prepared. In order to obtain the saturated sodium carbonate solution, 20 grams of sodium carbonate was weighed first and 20 ml of hot distilled water was added on it. The prepared solution was capped and boiled and dissolved thoroughly. Then, the temperature of the solution was cooled until it equaled to room temperature and 7 grams of sodium carbonate was added to the solution and the solution was made saturated. The saturated sodium carbonate solution we prepared was then incubated in the dark for 24 hours, filtered through the filter paper and pure water was added until the solution volume reached 100 ml. Samples were prepared to be analyzed in the next step. First, 2.4 ml of distilled water and then 40 µl of extract were put into glass tubes. 40 µl of methanol was added to control groups instead of extract. Then, 200 µl of folin, 600 µl of saturated Na_2CO_3 and 760 µl of distilled water

were added to the samples and mixed with the help of a vortex to completely dissolve the chemical substances in the mixture. After vortexed, the samples were kept at room temperature for 2 hours and absorbance was measured at 765 nm. Gallic acid was used for standard phenolic control. To prepare the gallic acid solution used in the study, firstly 3 mg of gallic acid was dissolved in 15 ml of methanol. Afterwards, control groups were prepared as 100, 125, 150, 175, 200 µg / ml by dilution and gallic acid curve was drawn. The samples were prepared in 4 replications and the spectrophotometric measurements for the determination of the phenolic content were carried out on the PerkinElmer Lambda 25 UV / VIS spectrophotometer.

4.4. Determination of Total Flavonoid Content

The total flavonoid content of the extracts were determined using the aluminum chloride colorimetric method of Biju et al. (Biju et al., 2014). 50 µl of the 1 mg / ml extract we prepared previously was drawn into a glass tube and 950 µl methanol was added. Then 4 ml of distilled water was added and vortexed to dissolve the mixture thoroughly. Then, 0.3 ml of 5% sodium nitrate ($NaNO_2$) was added and kept in the dark for 5 minutes. After the incubation process, 0.3 ml of 10% aluminum chloride ($AlCl_3$) was added and left in the dark again for 6 minutes. Then, 2 ml of 1 mole / L sodium hydroxide (NaOH) and 2.4 ml of distilled water were added and the solution was completed to 10 ml. After the solution obtained was kept in the dark for 15 minutes, absorbance was measured at 510 nm. In order to determine the quercetin standard, the main stock was prepared as 1 mg / ml and 6

different concentrations (10, 20, 40, 60, 80,100 µg / ml) were obtained by dilution. The total flavonoid substance content is indicated as mg quercetin equivalent (QE) / g extract. Each trial was made in 4 replications, and spectrophotometric measurements for the determination of the total flavonoid content were carried out with the PerkinElmer Lambda UV / VIS spectrophotometer device.

4.5. Statistical Analysis

All analyzes were done in four replications. The comparison of the extract yield, total phenolic and flavonoid content of the samples was made by the t-test. DPPH analysis results were evaluated by analysis of variance of LC 50 values and the differences between the averages with the Least Significant Difference (LSD) test. The obtained findings were given as mean ± standard deviation (SD), and the analyzes were carried out in the TARIST package program (Acikgoz et al., 2004).

5. RESULTS

In this study, the antioxidant capacity of *S. virgata* species grown in Yozgat ecological conditions, the leaves and flowers of the plant were used. The findings obtained from the plant parts used throughout the study are presented below.

5.1. Extract Yield

The extract amounts and extract yields of the samples were evaluated over 4 g for each sample. The amount of extract obtained from the

flowers was 0.1831 ± 0.236 and the extract yield was 4.4649 ± 0.5533%, while the extract amount obtained from the leaves was 0.2349 ± 0.207 and the extract yield was 5.7065 ± 0.4823%. When the data obtained as a result of the analysis are examined, it is seen that the amount of extract obtained from the leaves of *S. virgata* species and the extract yield are higher than that obtained from flowers. The observed difference was statistically significant at 1% level (Table 3).

Table 3: T-test for the extract yield

	Leaves	Flowers
Mean	5.707	4.460
Variance	0.233	0.307
Number of observations	3	3
Common variance	0.270	
SD	4	
t-calculated	2.989**	

**** Statistically significant at the 1% level

5.2. Antioxidant Activity

5.2.1. Total Phenolic Content

The total phenolic content of plant extracts was recorded as mg GAE / g extract. The total phenolic content of the extracts obtained from the flowers of the plant was found to be 50.6867 ± 5.3850 mg GAE / g, while the total phenolic content of the leaf extracts was found to be 50.1767 ± 8.7471 mg GAE / g.

As a result of the t-test, the difference between flowers and leaves of *S. virgata* grown in Yozgat ecological conditions was statistically not significant (Table 4)

Table 4: T-test for total phenolic content

	Leaves	Flowers
Mean	50.177	50.687
Variance	76.512	28.998
Number of observations	3	3
Common variance	52.755	
SD	4	
t-calculated	0.086 ns	

ns: statistically not significant

The absorbance value of the Gallic Acid Standard Curve at 765 nm of *S. virgata* grown in Yozgat ecological conditions ($R^2 = 0.996$) is given in Figure 4.

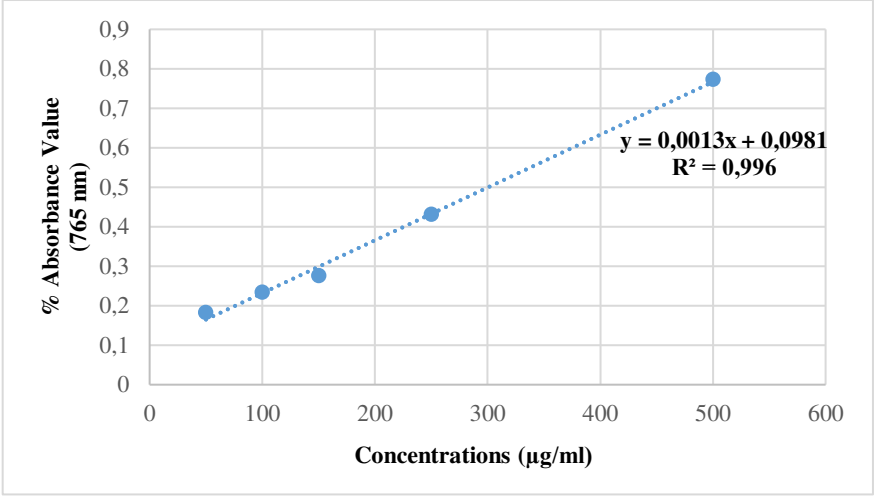

Figure 4: Gallic Acid Standard Curve

5.2.2. Total Flavonoid Content

The total flavonoid content of the extracts obtained from the flowers of *S. virgata* was determined as 121.4755 ± 11.6004 mg QE / g, while the total flavonoid capacity of the leaves extracts was determined as

72.6275 ± 8.7343. According to the t-test, the difference between the extracts was found to be statistically insignificant (Table 5).

Table 5: T-test for total flavonoid content

	Leaves	Flowers
Mean	72.627	121
Variance	76.265	1198.764
Number of observations	3	3
Common variance	637.514	
SD	4	
t-calculated	2.370 ns	

ns: statistically not significant

The absorbance values (R^2 = 0.9915) of the Quercetin Standard Curve of the extracts at 510 nm are given in Figure 5.

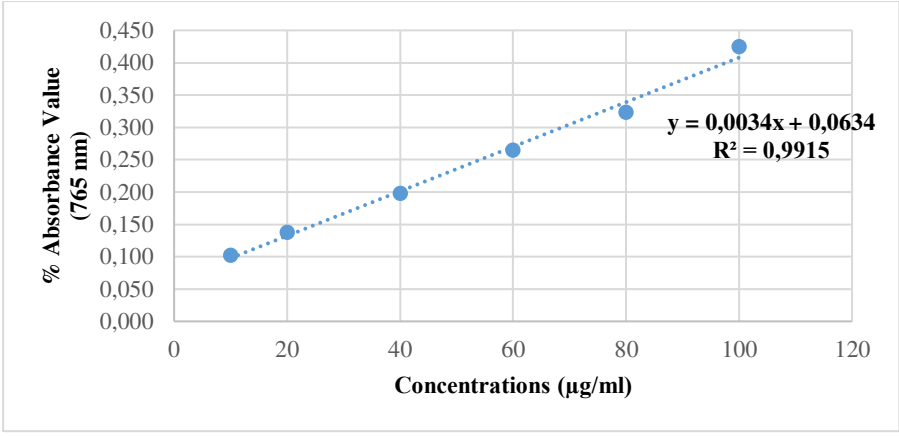

Figure 5: Quercetin standard curve

5.2.3. DPPH Radical Scavenging Activity

The IC_{50} value of the extracts obtained from the flowers of *S. virgata* was found to be 25.299 mg / ml, while the IC_{50} value of the extracts obtained from the leaves was calculated as 51.778 mg / ml. The total

amount of phenolic substance, total flavonoid substance and IC_{50} values found for each sample analyzed are given in Table 6.

Table 6: Total phenolic, flavonoid content and IC_{50} values of extracts obtained from the flowers and leaves of *S. virgata*

No	Sample	Total Phenolic (mg GAE/g)[a]	Total Flavonoid (mg QE/g)[b]	DPPH IC_{50} (mg/ml)[c]
1	Flower	50.6867±5.3850	121.4755±11.6004	25.299
2	Leaf	50.1767±8.7471	72.6275±8.7343	51.778

[a]GAE, gallic acid equivalent, [b]QE, quercetin equivalent, [c]IC_{50} values were expressed as mg/ml.

Table 7: Analysis of variance for DPPH values obtained from samples

Source of Variation	Degree of Freedom	Sum of Squares	Sum of Squares	F Value
Recurrence	2	1.330	0.665	0.541ns
DPPH (LC_{50})	3	2589.833	863.278	702.209**
Error	6	7.376	1.229	
General	11	2598.539	236.231	

ns: statistically insignificant, **: statistically significant at 1% level

According to the variance analysis results in Table 7, the factors were found to be statistically significant at 1% level.

Antioxidant activity increases as the DPPH LC_{50} value decreases, that is, antioxidant activity and antioxidant value are inversely proportional. Therefore, it was concluded that the antioxidant activity of the flowers extract of *S. virgata* plant grown in Yozgat ecological conditions is higher than the leaf extract. However, BHA and BHT used as controls exhibited higher antioxidant activity (Table 8, Figure 6).

Table 8: LSD grouping of DPPH values obtained from samples and standard antioxidants

DPPH (LC_{50})	**Mean**
Leaf	51.960 d
Flower	27.500 c
BHA	13.530 a
BHT	19.080 b

Mean square error:1.229 LSD ($_{0.05}$)=2.215

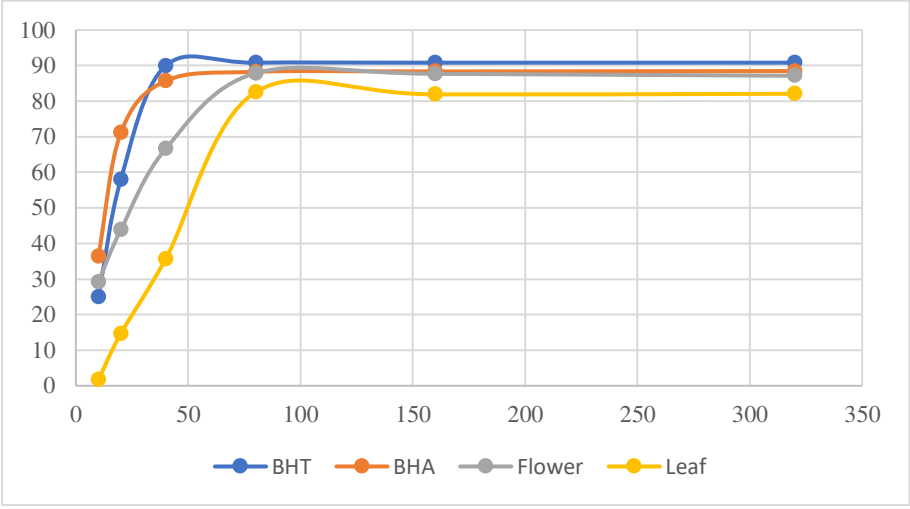

Figure 6: DPPH radical scavenging activities of the samples

6. DISCUSSION

Most of the medicinal and aromatic herbs rich in secondary metabolites have antioxidant effects. Phenols and flavonoids are generally determined plants antioxidant activity. (Baydar, 2013). Phenolic compounds are important compounds due to their effects on the quality characteristics of foodstuffs which are important in terms of consumption such as appearance, taste and flavor, and their positive

effects on human health as natural antioxidants (Nizamlioglu & Nas, 2010).

Free radicals cause damage cells and the immune system and accelerate aging. Antioxidants, on the other hand, bind free radicals to themselves or neutralize them, minimizing possible damage and thus delaying aging (anti-aging). Synthetic antioxidants such as PG (propyl gallate), TBHQ (tertiary butyl hydroquinone), BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are widely used in the food industry due to their greater stability than natural phenolic antioxidants. However, the use of these antioxidants has been limited in recent years due to their negative effects on human health. Because of that, the requisition for natural antioxidant sources is increasing daily (Mammadov, 2014).

The antioxidant activities of *Salvia* species and their total phenolic and flavonoid contents show a wide variation. The former studies have shown that the aerial parts of most Salvia species such as *S. officinalis*, *S. tomentosa*, *S verticillata*, *S. cryptantha*, *S. hypargeia*, *S. sclarea*, *S. russellii*, *S. virgata* and *S. ceratophylla* etc. exhibit strong antioxidant activity (Tepe et al., 2004; Tosun et al., 2009; Turtoglu et al., 2011; Orhan et al., 2013; Loizzo et al., 2014; Nickovar et al., 2016; Safaei-Ghomi et al., 2016). The total amount of phenolic substances in the extracts prepared using water and ethanol from the aerial parts of *S virgata* were determined as 120.14±2.27 and 195.22±0.25 mg GAE / g extract, respectively, while the total flavonoid contents of the same

extracts were recorded as 14.17±0.83 and 62.20±0.57 mg RE/g extract, respectively (Taghvaei & Jafari, 2015).

Generally, DPPH radical scavenging activities of extracts prepared with methanol were found to be higher (Taghvaei & Jafari, 2015; Karatoprak et al., 2016). Tosun et al. (2009) reported that the DPPH values of extracts obtained from the aerial parts of S. virgata and BHA were 23.4 µg/ml and 15.2 µg/ml, respectively. Similarly, DPPH values were found to be 65.70±2.12 µg mg^{-1} in plant extracts and 18.80±1.21 µg mg^{-1} in BHT in another study conducted by Tepe (2008). These results are similar to the findings we obtained from our study. As a matter of fact, the extracts used in our study exhibited lower antioxidant activity than synthetic antioxidants. However, contrary to these findings, there are studies reporting that S. virgata exhibits vary strong antioxidant activity (Dehghani Latani et al., 2019). The composition and number of phytochemicals with antioxidant activity such as phenolics and glucosinolates in medicinal plants vary according to many factors. Genetics, environmental conditions (amount of precipitation, altitude, soil conditions, temperature, etc.) physiological factors, cultural practices (harvest time, harvest period, irrigation, fertilization, etc.), used part of plants, extraction method and solvents used can affect the in vitro antioxidant activities of these compounds (Li et al., 2012; Balikci et al., 2018).

7. CONCLUSION

In this study, the flower parts of S virgata exhibited higher antioxidant activity than the leaves. Although the antioxidant activity of the extracts in our study is lower than the synthetic antioxidants, it has been observed in the literature that the plant has a strong antioxidant activity. In this context, extracts from this type have the potential to be used in industry. Therefore, detailed studies are needed to determine the components in different parts of this species (flower, leaf, root, etc.) and to evaluate their antioxidant activities.

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CHAPTER 8

THE CARYOLOGICAL STUDIES ON *Salvia sclarea* L., *Salvia aethiopis* L. AND *Salvia verticillata* subsp. *amasiaca* (Freyn & Bornm.) IN TURKEY

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INTRODUCTION

Lamiaceae is one of the most important family all over the world, they are especially used in industrial area such as medicine, food, cosmetics and perfumery. This family includes 224 genus and 5600 species in the world. Additionally, they are found in the flora of Turkey 725 taxa (subspecies, varieties and hybrids) belonging to 45 genus and 565 species of which 1/3 are endemic (Davis, 1982; Dweck, 2000). The genus *Salvia*, which comprises more than 95 species (51% endemic) in Turkey, is one of the most important members of the Lamiaceae family. In flora of Turkey, 97 species grow in the Mediterranean (27.8%) and the Euro-Siberian phytogeographic regions (5%), but many are found around the Iran-Turan (59.7%) regions (Poyraz & Koca, 2006; Celep, 2009). According to the genetic diversity of these plants with such a wide spread, different levels such as genus, species, community, genome, gene location and DNA series can be examined. In addition to morphological and biochemical qualifications, karyotype differences, lysozymes and DNA-based markers and markers are used in the classification of interspecific and in determining interspecies diversity. Changes in chromosome structure and number are become valuable as a source of distinctive genetic markers interspecies (White, 1973). Cytogenetic findings allow revealing differences and similarities that cannot be observed morphologically (Hillis & Moritz, 1990; Gosden, 1994). Chromosomal characters are used to elucidate phylogenetic relationships in plant cytotaxonomy (Eroglu et al., 2020).

The relationships between ploidy levels, chromosome numbers and geographical distribution indicate that both aneuploidy and polyploidy have played an important role in the speciation processes in Salvia (Ranjbar et al., 2015).

In a cytomorphological study on some taxa of *S. hypoleuca*, *S. ceratophylla,* *S. limbata* and *S. sclarea*, *S. staminea* and *S. xanthocheila* were reported as 2n=22 chromosome number (Özdemir and Senel 1999; Martin et al., 2015), *S. verticillata* and *S. verticillata* subsp. *amasiaca* were showed 2n=4x=32 (Lövkvist and Hultgård , 1999; Martin et al., 2015). According to the Ozkan et al. (2017), it has been observed that *Salvia aethiopis* has a chromosome number equivalent of 2n=2x=22.

The aim of this study is to provide chromosomal data for this gene pool of the Salvia genus. The species studied are *S. sclarea* L., *S. aethiopis* L. and *S. verticillata* L. subsp. *amasiaca* (Freyn & Bornm.). According to this study chromosome counts and all the karyotype patterns have been conducted. Some of the counts confirm those contained in previous reports and some are different.

1. MATERIAL AND METHOD

1.1. Plant Material

Salvia species were collected from their natural habitats across Yozgat, Turkey. The plant samples were deposited at the herbarium of the Department of Field Crops, at the Yozgat Bozok University in Yozgat. The collection information is given below.

***Salvia sclarea* L.**: Turkey, Yozgat, Akdağmadeni, Yıldızeli road, 39˚45´39.55´´N; 35˚55´45.5´´E, 1160 m. ***Salvia aethiopis* L.**: Turkey, Yozgat, Akdağmadeni, Sorgun road, 39˚41´32.03´´N; 35˚23´46.20´´E, 1130 m ***S. verticillata* L. subsp. *amasiaca* (Freyn & Bornm.):** Turkey, Yozgat, Akdağmadeni, Akdağmadeni road, 39˚41´25.72´´N; 35˚43´47.49´´E, 1220 m.

1.2. Chromosome Preparation

Salvia seeds were germinated between moist Whatman papers in Petri dishes. The root tips were pretreated in α-mono-bromonaphthalene at 4°C for 16 h. Then, the roots were fixed by Carnoy's fixative (ethyl alcohol: acetic acid - 3:1, v:v) at 4°C for 24 h and stored in 70% ethyl alcohol at 4°C until use. Then, the fixed roots were hydrolyzed in 1 N HCl at 60°C for 12 min, stained in 2% aceto-orcein, and squashed for observations (Eroğlu et al.,2020; Martin et al., 2020).

1.3. Karyotype Analysis

At least ten mitotic cells were observed to identify diploid chromosome numbers. The chromosomal measurements were made using the KaryoType software (Altınordu et al., 2016) loaded on a personal computer. The following parameters were used to characterize the chromosomes numerically (Table 1). According to Levan et al. (1964) chromosome morphology based on centromere position were by karyotype formulae. The ideograms were drawn based on chromosome arm length (arranged large to small). In Table 1, karyotype asymmetries

were estimated by many different parameters as the mainly interchromosomal asymmetry (CV_{CL}) and intrachromosomal asymmetry (M_{CA}) (Paszko, 2006; Peruzzi & Eroğlu; 2013).

Table 1: The chromosomal parameters and formulae.

Chromosomal Parameters	**Formulae and Abbreviations**
Short Arm Length	S
Long Arm Length	L
Total Chromosome Length	TCL = SA + LA
Arm Ratio	AR = LA / SA
Centromeric Index	CI = [(SA) / (TCL)] × 100
Total Haploid Length	THL
Mean Haploid Length	MHL
Relative Length	RL = [(TCL) / THL] × 100
Metacentric Chromosome Submetacentric Chromosome Subtelocentric Chromosome Telocentric Chromosome	m, AR = 1.0 – 1.7 sm, AR = 1.7 – 3.0 st, AR = 3.0 – 7.0 t, AR = 7.0 – ∞
Intrachromosomal Asymmetry Mean Centromeric Asymmetry	M_{CA} = [mean (L_T – S_T) / (L_T + S_T)] × 100 L_T (Total Length of Long Arms) S_T (Total Length of Short Arms)
Interchromosomal Asymmetry Coefficient Variation of Chromosome Length	CV_{CL} = (S_{CL} / X_{CL}) × 100 S_{CL} (Standard Deviation) X_{CL} (Mean Chromosome Length)

2. RESULTS

Diploid chromosome numbers of *S. sclarea, S. aethiopis, and S. verticillata* were determined as $2n = 2x = 22$, 22 and 30, respectively (Table 2).

Table 2: Karyological features and karyotype asymmetries of studied *Salvia* species.

Parameters	***Salvia sclarea***	***Salvia aethiopis***	***Salvia verticillata***
2*n*	22	22	30
KF	20m + 2sm	18m + 4sm	26m + 4sm
SC (µm)	1.66	1.24	0.85
LC (µm)	2.73	2.07	2.56
RL (min–max)	7.25–11.92	6.90–11.52	3.57–10.74
THL (µm)	22.90	17.97	23.84
MHL (µm)	2.08	1.63	1.59
CI (min–max)	34.69–47.09	32.45–48.42	34.38–47.31
CV_{CL}	16.59	16.97	25.36
M_{CA}	15.03	18.30	14.47
AsK (%)	57.60	59.38	57.63
TF (%)	42.40	40.62	42.37
Syi (%)	73.62	68.42	73.51
Rec (%)	76.26	78.92	62.08
A1	0.26	0.30	0.24
A2	0.17	0.17	0.25
A	0.15	0.18	0.14
DI	6.94	6.85	10.98
AI	1.39	1.98	2.62

Abbreviations: shortest chromosome length (SC); karyotype formula (KF); longest chromosome length (LC); relative length (RL); total haploid chromosome length (THL); mean chromosome length (MHL); coefficient of variation of chromosome length (CV_{CL}); mean centromeric asymmetry (M_{CA}); centromeric index (CI); karyotype asymmetry index (AsK); total form percent (TF); index of chromosomal size resemblance (Rec); index of karyotype symmetry (Syi); intrachromosomal asymmetry index (A1); interchromosomal asymmetry index (A2); degree of karyotype asymmetry (A); dispersion index (DI); asymmetry index (AI).

2.1. *Salvia sclarea*

The chromosome number of *S. sclarea s* is *2n* = 22 (Figure 1). The karyotype formula is 20m + 2sm. The ideogram is given in Figure 2. *S. sclarea* chromosome lengths are between 1.66 and 2.73 µm. Total and mean haploid lengths are 22.90 and 2.08 µm, respectively (Table 3). The values of karyotype asymmetry indexes for the intrachromosomal and interchromosomal are 15.03 and 16.59 for MCA and CVCL, respectively.

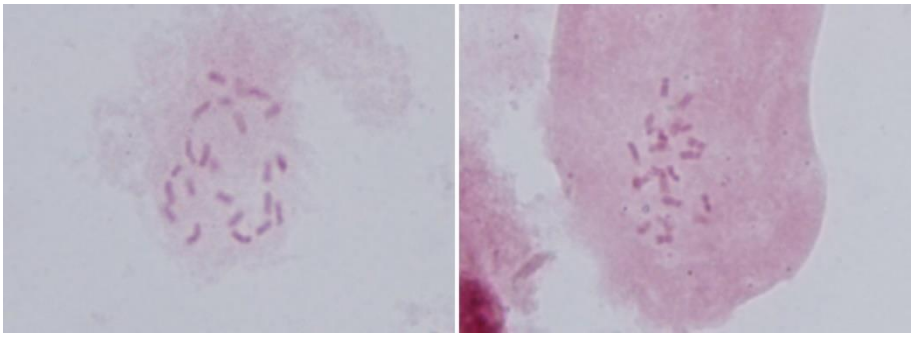

Figure 1: The metaphase chromosomes of *Salvia sclarea*.

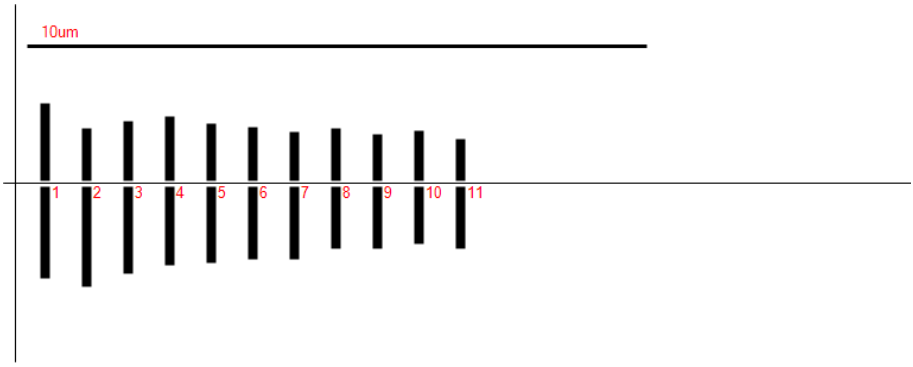

Figure 2: The monoploid ideogram of *Salvia sclarea*.

Table 3: The detailed chromosomal measurements of *Salvia sclarea*.

Pair	L + S (μm)	L (μm)	S (μm)	L / S	RL (%)	CI (%)	Type
1	2.73	1.48	1.25	1.18	11.92	45.79	m
2	2.45	1.60	0.85	1.88	10.70	34.69	sm
3	2.36	1.40	0.96	1.46	10.31	40.68	m
4	2.30	1.26	1.04	1.21	10.04	45.22	m
5	2.13	1.22	0.91	1.34	9.30	42.72	m
6	2.03	1.17	0.86	1.36	8.86	42.36	m
7	1.95	1.17	0.78	1.50	8.52	40.00	m
8	1.83	0.99	0.84	1.18	7.99	45.90	m
9	1.74	1.00	0.74	1.35	7.60	42.53	m
10	1.72	0.91	0.81	1.12	7.51	47.09	m
11	1.66	0.99	0.67	1.48	7.25	40.36	m

2.2. *Salvia aethiopis*

The chromosome number of *S. aethiopis s* is *2n* = 22 (Figure 3). The karyotype formula is 18m + 4sm. The ideogram is given in Figure 4. The chromosome lengths are between 1.24 and 2.07 μm. Total and mean haploid lengths are 17.97 and 1.63 μm, respectively (Table 4). The values of karyotype asymmetry indexes for intrachromosomal and interchromosomal are 18.30 and 16.97 for M_{CA} and CV_{CL}, respectively.

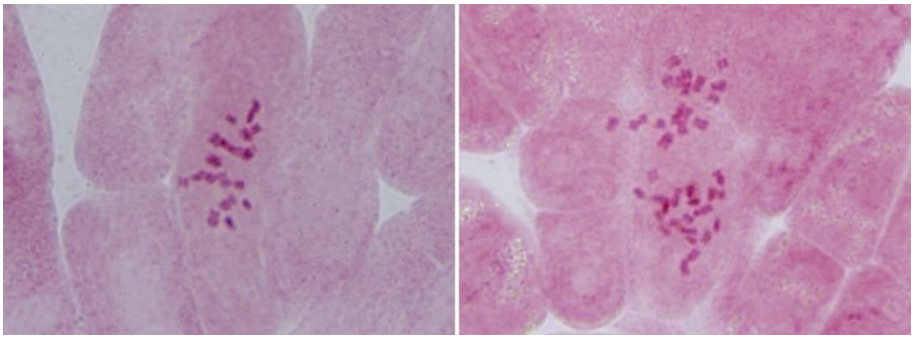

Figure 3: The metaphase chromosomes of *Salvia aethiopis.*

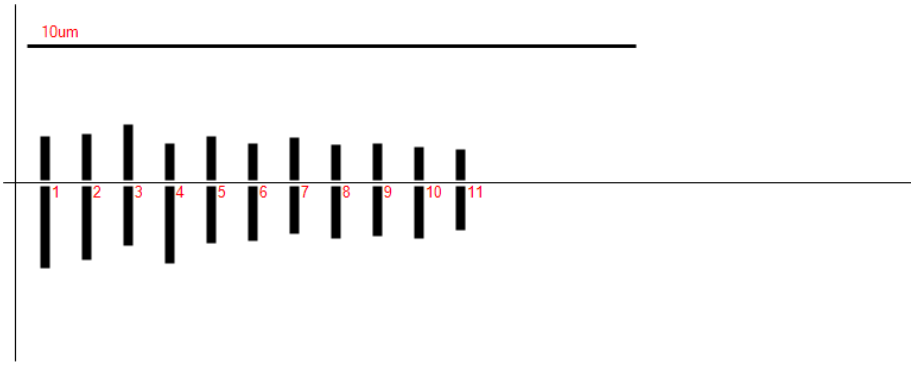

Figure 4: The monoploid ideogram of *Salvia aethiopis*.

Table 4: The detailed chromosomal measurements of *Salvia aethiopis*.

Pair	L + S (µm)	L (µm)	S (µm)	L / S	RL (%)	CI (%)	Type
1	2.07	1.34	0.73	1.84	11.52	35.27	sm
2	1.97	1.21	0.76	1.59	10.96	38.58	m
3	1.90	0.98	0.92	1.07	10.57	48.42	m
4	1.88	1.27	0.61	2.08	10.46	32.45	sm
5	1.66	0.94	0.72	1.31	9.24	43.37	m
6	1.51	0.90	0.61	1.48	8.40	40.40	m
7	1.49	0.78	0.71	1.10	8.29	47.65	m
8	1.43	0.85	0.58	1.47	7.96	40.56	m
9	1.42	0.81	0.61	1.33	7.90	42.96	m
10	1.40	0.86	0.54	1.59	7.79	38.57	m
11	1.24	0.73	0.51	1.43	6.90	41.13	m

2.3. *S. verticillata* L. subsp. *amasiaca* (Freyn & Bornm.)

S. verticillata L. subsp. *amasiaca*'s chromosome number is *2n* = 30 (Figure 5). The karyotype formula is 26m + 4sm. The ideogram is given in Figure 6. The chromosome lengths are between 0.85 and 2.56 µm.

Total and mean haploid lengths are 23.84 and 1.59 µm, respectively (Table 5). The values of karyotype asymmetry indexes for the intrachromosomal and interchromosomal are 14.47 and 25.36 for M_{CA} and CV_{CL}, respectively.

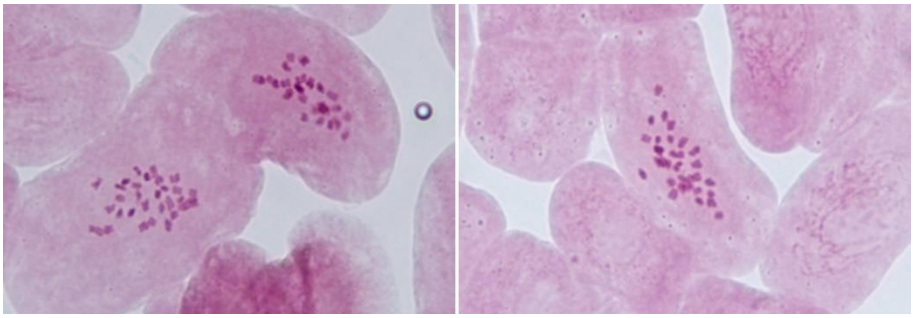

Figure 5: The metaphase chromosomes of *Salvia verticillata.*

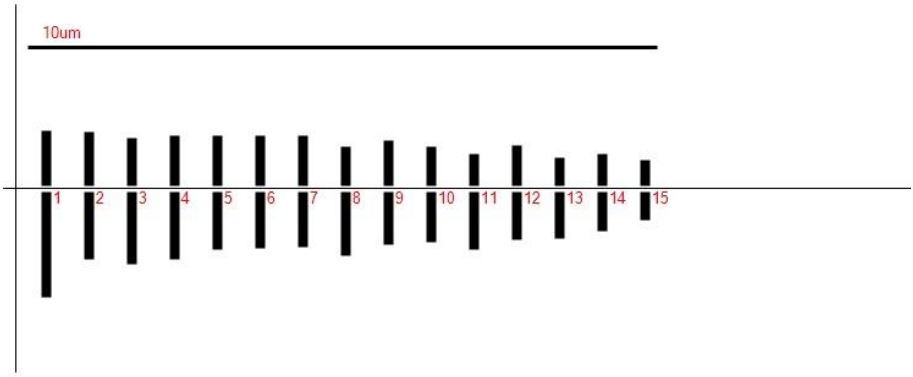

Figure 6: The monoploid ideogram of *Salvia verticillata*.

Table 5: The detailed chromosomal measurements of *Salvia verticillata*.

Pair	L + S (μm)	L (μm)	S (μm)	L / S	RL (%)	CI (%)	Type
1	2.56	1.68	0.88	1.91	10.74	34.38	sm
2	1.91	1.06	0.85	1.25	8.01	44.50	m
3	1.90	1.15	0.75	1.53	7.97	39.47	m
4	1.85	1.06	0.79	1.34	7.76	42.70	m
5	1.70	0.91	0.79	1.15	7.13	46.47	m
6	1.69	0.90	0.79	1.14	7.09	46.75	m
7	1.67	0.88	0.79	1.11	7.00	47.31	m
8	1.64	1.01	0.63	1.60	6.88	38.41	m
9	1.54	0.83	0.71	1.17	6.46	46.10	m
10	1.42	0.79	0.63	1.25	5.96	44.37	m
11	1.41	0.91	0.50	1.82	5.91	35.46	sm
12	1.40	0.76	0.64	1.19	5.87	45.71	m
13	1.18	0.73	0.45	1.62	4.95	38.14	m
14	1.12	0.62	0.50	1.24	4.70	44.64	m
15	0.85	0.45	0.40	1.12	3.57	47.06	m

3. DISCUSSION

The karyological characters as especially diploid chromosome size, chromosome number and chromosome symmetry/asymmetry are preferred parameters in plant cytotaxonomy. In addition, the data are important to elucidate the origin, speciation and interspecific relationships of plants (Eroğlu et al., 2013). The chromosome numbers of *Salvia sclarea, S. aethiopis,* and *S. verticillata* are $2n$ = 22, 22 and 30, respectively. The species have small chromosomes between 0.85–2.73 μm. Chromosomes are represented little variations in size.

There are many *Salvia* species reported chromosome numbers (Rice et al., 2015). In genus, it was reported that the diploid numbers are $2n$ = 22 in *S. sclarea* (Rosúa & Blanca, 1988; Murin, 1997), $2n$ = 22, 22+(0-

2)B and 24 in *S. aethiopis* (Markova & Ivanova, 1982; Rosúa & Blanca, 1988), and $2n$ = 16, 32 in *S. verticillata* (Patudin et al., 1975). Accordingly, there are both similarities and differences to the study results.

In genus *Salvia*, there are many basic numbers, which are 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, and 21. In basic numbers, infraspecific variations are encountered in genus *Salvia*. In the present study, the basic numbers and ploidy levels are x = 11 in *S. sclarea* and *S. aethiopis* with ploidy level of $2x$ and x = 15 in *S. verticillata* with ploidy level of $2x$.

Interchromosomal asymmetry is determined by CV_{CL} parameter, which is varies from 0 (no variation) to 100 (Paszko, 2006). The CV_{CL} values of *Salvia sclarea, S. aethiopis* and *S. verticillata* are 16.59, 16.97 and 25.36, respectively. Intrachromosomal asymmetry is determined by M_{CA} parameter, which is varies from 0 (perfectly symmetric) to 100 (perfectly asymmetric) (Peruzzi & Eroğlu, 2013). The M_{CA} values of *S. sclarea, S. aethiopis* and *S. verticillata* are 15.03, 18.30 and 14.47, respectively, which refer to symmetric karyotypes. Symmetric karyotypes are defined by metacentric and submetacentric chromosomes. All species have metacentric and submetacentric chromosomes, whereas no subtelocentric and telocentric chromosomes. Centromere position changes in intracromosomal asymmetry. In addition, the sizes of small and large chromosomes are quite different in interchromosomal asymmetry (Peruzzi et al., 2009).

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CHAPTER 9

BIO-FERTILIZERS EFFECTS ON QUALITATIVE AND BIOCHEMICAL PROPERTIES OF DENAYI THYME (*Thymus daenensis subsp. daenensis* Celak)

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INTRODUCTION

The genus *Thymus* L. belonging to the botanical family of Lamiaceae, and consists of about 250 species of small shrubs and herbaceous perennials all over the world. The center of the genus has been identified in Mediterranean region (Manzoor et al., 2018). The aerial parts and volatile constituents of the plant are commonly used as medicinal herb. Thymus species are commonly used as herbal tea, flavoring agents, dyeing, and for medicinal purposes infusion and tonic, carminative, digestive, antispasmodic, anti-inflammatory, and expectorant and for the treatment of colds in traditional medicine. The aromatic and medicinal properties of the genus Thymus has made it one of the most popular medicinal plants (Sun et al., 2015). It is believed that these characteristics are to some extent caused by the constituents. The genus Thymus has numerous species and varieties and their essential oil composition has been studied earlier (Majdoub et al., 2017). The genus Thymus one of the most important herb has considered as an economically and commercial herb, native to Southern Europe, and with a worldwide distribution (Baghaie et al., 2019). There are considerable research interests in studying compositional analysis of Thymus essential oil and its extract. It is well known that yield and yield components of plants are determined by a series of factors including plant genetic, climate, edaphic, elevation, and topography and also an interaction of various factors (Padash et al., 2019). Denayi Thyme (*Thymus daenensis* subsp**.** *daenensis* Celak) as most popular species of thyme has different pharmacological properties, including

anti-viral, anti-bacterial, anti-fungal, antioxidant, insecticidal and immunomodulatory. The aromatic profile of the species is characterized by phenols, aromatic and non-aromatic monoterpenes such as thymol and carvacrol and their biosynthetic precursor's p-cymene and γ-terpinene, respectively. These components not only are responsible for the aroma and flavor of the herb but also significantly contribute to its biological effects (Wasli et al., 2018). So, the objective of this research was to determine the growth, yield and phytochemical composition of Denayi thyme under Urmia ecological condition as influenced by the application of various biofertilizers.

Large amounts of chemical fertilizers have been applied into arable fields over the past few decades in order to maximize the crop yields and prevent food shortage worldwide. However, excessive use of chemical fertilizers can cause serious soil degradation such as nitrogen leaching, soil compaction and reduction in soil organic matter; and consequently, the efficacy of chemical fertilizers on crop yields decreases over time (Lajayer et al., 2019). Indiscriminate use of chemicals and fertilizers has altered the biological ecosystem, affected non-target organisms and adversely influenced microorganisms in the soil (Fattahi et al., 2019). Organic farming, which aims at cultivating the land and raising crops in such a way to keep the soil alive and in good health, may be an alternative to the present system of farming which solely depends on chemicals. Recently, a great attention was paid towards the application of bio-organic farming to avoid the heavy use of agrochemical that resulted in numerous of environmental troubles

(Seyedalikhani et al., 2019). The coincident application of organic manures and bio-fertilizers is frequently recommended for improving soil properties and obtaining clean agricultural products. Bio-fertilizers are commonly called as microbial inoculants which are capable of mobilizing important nutritional elements in the soil from non-usable to usable form by the crop plants through their biological processes. For the last one-decade, bio-fertilizers (especially nitrogen and potash fertilizers) are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere (Wasli et al., 2018). Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants. Such strophic associations are of significance. The plant growth promoting rhizobacteria (PGPRs) can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of biocontrol agents against soil-borne phytopathogenes (Barouchas et al., 2019). Medicinal plants have an important value in the socio-culture, spiritual and medicinal use in rural and tribal lives of the developing countries (Caunii et al., 2015). Recently, the production of chemical-free medicinal and aromatic plants has been the focus of interest of many researchers and producers in order to ensure the high quality and safety, not only for human, but also for the environment (Seyedalikhani et al., 2019). Investigation took place for using bio-fertilizers as an alternative to chemical fertilizers or at least minimizes the levels of these chemicals in order to protect the environment from pollution, decrease the production cost and produce chemical free

product ((Baghaie et al., 2019). Bio-fertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. These potential of biological fertilizers would play the key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers (Lajayer et al., 2019).

1. MATERIAL AND METHOD

1.1. Field Experiment

The trial was done at the experimental fields of Urmia University, Urmia, West Azerbaijan, Iran (Lat. 37°31' N., Long. 45°02' E., Alt. 1320 m.) in the 2018-2019 growing season. The experimental land was plowed at the optimum moisture level (field capacity) and leveled. Sowing were done in an open field at the experimental fields of the Department of Plant Production and Genetics, Faculty of Agriculture, Urmia University. The mean annual rainfall and temperature were shown in Figure 1.

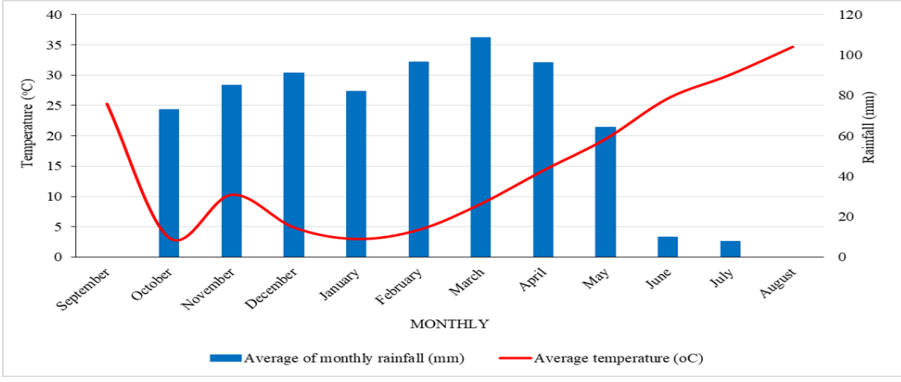

Figure 1. Climatic data of the experiment city (Rahimi et al., 2019)

1.2. Soil Analysis

Soil properties were determined according to methods given in Mahdi (2016). Soil pH was measured using 1:5 soil to water ratio suspension with a glass electrode pH meter (model Inolab pH 7110). Soil electrical conductivity (EC) was measured using a glass electrode (model 712 conductometer) after mixing the soil with water (1:5, w/v). Organic matter (OM) was determined according to the Walky-Black method, which is based on the oxidation of soil organic fraction with $K_2Cr_2O_7$ and H_2SO_4 and titration with $FeSO_4$. Cation exchange capacity (CEC) was measured by saturation the soil with 1 mol L^{-1} sodium acetate solution (pH 8.2), washing soil soluble sodium (Na^+) with 96% ethanol and extracting exchangeable Na^+ with 1 mol L^{-1} of neutral ammonium acetate. Calcium carbonate ($CaCO_3$) was determined after boiling of 2.5 g soil with 25 ml of 0.5 N HCl and titration with 0.25 N NaOH. Three fractions (sand, silt, and clay) of soil particles were determined following the pipette method (Rowell, 1994).

Selected chemical and physical properties of the five studied soils are shown in Table 1.

Table 1: Mean physiochemical properties of studied soil

pH	EC (dS m^{-1})	OC (%)	Olsen-P (mg kg^{-1})	Available-K (mg kg^{-1})	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)
7.33	0.066	1.14	37.60	166	9.0	44	33	24

According to the obtained pH, soil classified as neutral (ranging from 6.5 to 7.5) but it is not alkaline soil due to the low EC (less than 2 dS m^{-1}). As well as, relatively high organic carbon (1.14 %) and loamy texture of studies soil samples relevant the appropriate conditions was

performed for growing of the plant Based on soil nutrients analysis, no fertilization was necessary in studied soils (P and K concentrations more than 15 mg kg^{-1} and 60 mg kg^{-1} respectively). The use of organic manures and chemical fertilizers in Iranian agricultural farms is more than the needs of plants and soil and water research institute recommendations, which leads to the accumulation of organic and inorganic compounds in soil and their decomposition over time (Barouchas et al., 2019).

The field trial was carried out as complete block design with five treatments and three replications. The seeds (populations) for sowing were obtained from Isfahan Agricultural Research Center. Sowing was carried out in green house at the green house of the Department of Horticulture during the period from 21. 03. 2018 till 06.05.2019. The seeds were sowed in plastic pots filled with soil, sand, and peat moss substrate as a material to germination. After sowing was irrigated regularly depending on weather conditions and development stage of plants. Seedlings were harvested and planted in the field. Seedlings of the plant with a height of 15 cm planted on 21 July in a plant spacing of 50 × 30 cm. For biofertilization a soluble of each biofertilizer with tap water is provided and sprayed to roots as recommended by the company. The seedlings were sown after inoculation with the biofertilizers. Biofertilizer at five levels (Azotobacter; Azotobacter + manure; Azotobacter + vermicompost; Azotobacter + fertile phosphate 2; Azotobacter + chemical fertilizer; and control). Azotobacter contains the bacteria of the O_4 strain of Azotobacter *vinelandii,* which fixes

atmospheric N actively into the forms that are absorbable by plants. One 100-g container of Azotobacter can be an effective replacement for 30-50 kg chemical N fertilizer. The phosphate biofertilizer contained two phosphate-solubilizing bacteria that decompose insoluble phosphorus compounds of soil by two mechanisms - the secretion of organic acids and enzyme phosphatase. Then, this nutrient becomes available to plants. Based on the amount of soil absorbable phosphorus, each package of this biofertilizer can replace 50-100% of the chemical phosphate fertilizer demand of plants. The biofertilizer Pota-Barvar-2 contains two potassium solubilizing bacteria that decompose insoluble potassium in the root zone and release its ions, thereby optimizing potassium uptake. So, it can be a replacement for at least 50% of potassium chemical fertilizers. Seedlings were irrigated immediately after planting and a weekly irrigation interval was used. Weeds were controlled by hand when needed. Growth parameters were recorded adjust before harvesting. For this purpose, five plants were randomly selected from each treatment plot. Plants were collected at full flowering stage (Figure 2). Collected materials were weighted before and after drying. Dried materials were sent to the laboratory for further phytochemical study.

Figure 2. The cultivated *Thymus daenensis* subsp**.** *daenensis* Celak

1.3. Plant Growth Characteristics

After harvesting the samples, characteristics such as leaf dry weight per plant (g), stem dry weight per plant (g), total dry weight per plant (g), (%) were measured.

The content of Nitrogen (N), phosphorus (P), potassium (K), Iron (Fe), zinc (Zn), and copper (Cu) in plant leaf were determined according to Lajayer et al (2019).

1.4. Super Oxide Radical Scavenging Activity

To measure superoxide anion radicals, superoxide anion radicals were generated by a pyrogallol autoxidation system. The test tube containing 9 ml of Tris buffered saline (pH = 8.2, 50 mmol / l) was incubated for 20 minutes in a mortar at 25° C. 40 microliters of pyrogallol solution (45 mmol / l pyrogallol in 10 mmol hydrochloric acid), previously incubated at 25° C, was injected into the upper part of the test tube using a microliter syringe. And it was mixed. The mixture was incubated at 25° C for 3 minutes and then 1 drop of ascorbic acid (0.035%) was

immediately inoculated to complete the reaction. The adsorption of the mixture at 420 nm was recorded as A_0 after 5 min, and this A0 shows the rate of pyrogallol autoxidation. The A1 autoxidation rate was increased by the same method only with a certain amount of extract (10 μL) in Tris buffer. At the same time, a control blank of reactive materials was considered as A2. The percentage of radical accumulation was calculated using the following formula (Caunii et al., 2015):

Super oxide radical scavenging (%) = $[(A_0-A_1/A_0)] \times 100$ Eq. (1)

Where A_0 is theabsorbance of the Tris-HCl buffer with pyrogallol, A_1 is the absorbance of the extract addition.

1.5. Nitric Oxide Radical Scavenging Activity

Nitric oxide radical inhibition was calculated using Griess Illosvoy reaction. In this method, the Griess Illosvoy reaction agent was modified by substituting naphthylene diethylamide dihydrochloride (0.1% volume / weight) instead of 1-naphthylamine (5%). 3 ml of the reaction solution was incubated with 2 ml of sodium nitroprusside (10 mM), 0.5 ml of saline phosphate buffer, and 40 ml of the plant extract for 25 minutes at 25° C. After incubation, 0.5 ml of the resulting solution was mixed with 1 ml of sulfanilic acid (0.33% in 10% glacial acetic acid) and allowed to stand for 5 min to complete permanent denaturation. Then 1 ml of naphthylethylenediamine dihydrochloride was added to the mixture and allowed to stand for 30 minutes at 25° C. A diffuse pink color appeared in the light background. The absorbance of this solution was read at 540 nm against a blank. The percentage of

nitric oxide radical accumulation was calculated using the following formula (Caunii et al., 2015):

$$\text{Nitric oxide radical inhibition } (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{Eq. (2)}$$

Where $A_{control}$ is absorbance of control sample and A_{sample} absorbance in the presence of the samples of extracts or standards.

1.6. Total Phenolic Content (TPC)

The total phenol content of the extracts was determined using Folin-Ciocalteu and Hurwitz (1984) method with slight modification. According to this method, 1 ml of Folin-Ciocalteu (diluted 1:10) was added to 50 ml of the plant extract. Then the solution was mixed with 1 ml of sodium carbonate (10%) and they were incubated at room temperature and dark for 60 minutes. Finally, the absorbance of the solution was measured using a spectrophotometer at 750 nm. Total phenolic content was expressed in mg kg^{-1} of gallic acid in 100 g of extract using standard gallic acid curve.

1.7. Preparation of Methanol Extract

The Adebayo and Ishola (2009a) method of extraction was used. 250 g of the plant part (leaf) were packed in a soxhlet extractor and extracted with methanol. The methanol extracts were evaporated to dryness using a rotary evaporator (Stuart, Barloworld and Model RE 300). The micronutrient uptake of the extract was read by atomic absorption

spectrophotometer using elemental standards and reported in mg kg^{-1} according to Caunii et al., 2015.

1.8. Essential Oil Percentage

The essential oil was extracted by the method of distillation with water and using a Clevenger. Then, essential oil percentage was estimated by the weight method (Caunii et al., 2015).

1.9. Statistical Method

All experimental sections were performed in triplicate, results were expressed as mean ± SE. Analysis of variance was performed by ANOVA procedure, and significant differences were calculated according to Duncan's multiple range tests ($p < 0.05$) using SAS (version 9.1.3) software.

2. RESULTS AND DISCUSSION

The effect of various bio-fertilizers on some plant growth parameters are shown in Figure 3.

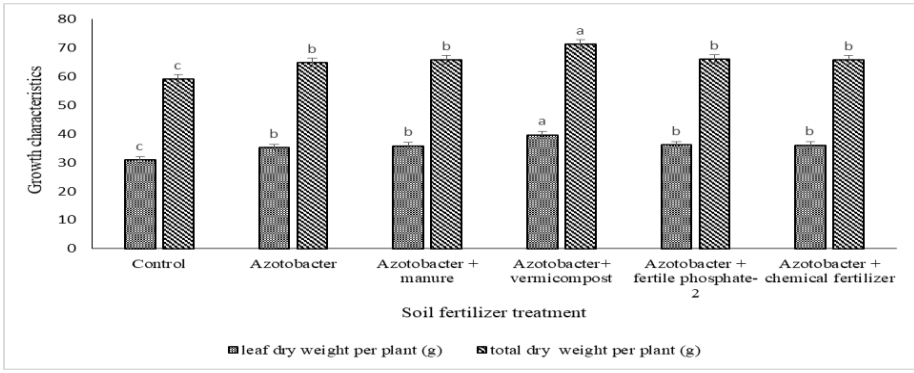

Figure 3. Effect of different bio-fertilizers on some properties of Denayi thyme

The results of ANOVA showed that simple effects of fertilizing systems was significant on plant growth parameters at 5% levels (Table 2, Figure 3). The simple effects of fertilizing systems showed a significant increase in leaf dry weight, and total dry weight for chemical fertilizer compared to the control. The highest leaf dry weight per plant and total dry weight were found Azotobacter+ vermicompost application. The integrated fertilizer provided the possibility of absorption of essential nutrients in early stages of growth. In the vegetative growth stages, animal manure provided more micro and macro nutrients to support better performance of the plants (Samavatipour et al., 2019).

Table 2: Analysis of variance of different parameters for *Thymus daenensis* Celak as influenced by biofertilizers

Variation	Leaf dry weight (g)	Stem dry weight (g)	Total dry weight (g)	Total phenol (mg GAE g^{-1} DW)	Scavenging Superoxide (%)	Scavenging Nitric oxide (%)	Essential oil (%)
Block	0.74	0.44	0.82	0.22	0.88	0.95	0.07
Treatment	6.94**	0.52ns	6.06 ns	18.44**	10.29**	11.20**	1.99ns
Error	3.33	6.73	7.31	2.81	4.90	5.29	0.008
CV (%)	5.11	8.69	4.12	3.86	5.55	7.12	2.83

The results of a study on chamomile showed that biofertilizers application could result in higher competition among neighboring plants for light. Higher planting density not only resulted in no beneficial effects on the final size of the plants, but it also decreased the qualitative and quantitative plant characteristics (Ghasemi Pirbalouti et al., 2013). Pisoschi et al. (2016) observed that foliar application of amino acids increase plant growth parameters in celeriac. Lajayer et al (2019) also reported the enhancement of plant growth parameters in *Matricaria chamomilla* as the result of biological promoter application. These finding were also observed in the experiments of Espanany et al. (2016) on *Calendula officinalis* L. and Singleton et al (1999) on *Descurainia Sophia*. Zahedifar et al. (2019) reported that the maximum impact of biofertilizers application was seen in the leaf and root dry weight, while it's least impact was noticed in fresh root weight among the studied traits. They explained that biofertilizers increased the growth of some root traits such as length, number, fresh and dry weights as well as the volume. The results of this study on biofertilizers application correspond with the results of Caunii et al. (2015) on wheat.

No significant effects of planting density, fertilizer treatments or their interactions were observed in chlorophylls a or b. Okpashi et al. (2019) indicated that increased plant density decreased the photosynthesis rate because the increased leaf surface area caused more shade on the lower leaves via decreasing light absorption efficiency.

The photochemical compounds in plants are considered to be antioxidants that have similar antioxidant capacity to synthetic antioxidants without side effects (Esmielpour et al., 2016). The effect of different treatments on radical scavenging activity is shown in Figure 4.

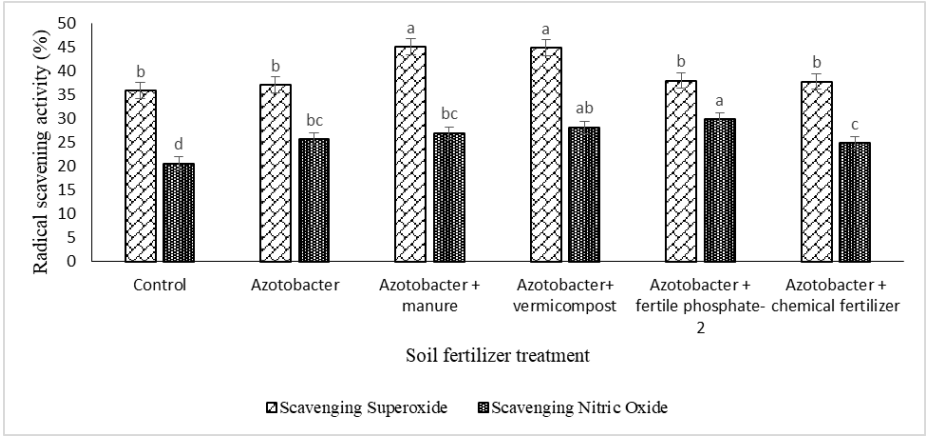

Figure 4. Effect of various bio-fertilizers on radical scavenging activities of Denayi thyme

Antioxidants exist in both natural and synthetic forms. In recent years, the use of synthetic antioxidants such as TBHQ, BHT and BHA like other chemical additives has been limited due to their potential toxicity and carcinogenicity. Nowadays most of the research is done on using new antioxidants without risk from plant sources, animal, microbial and

food are concentrated (Zahedifar et al., 2019). Research on ginger showed that under conditions of improved soil properties and as a result of increased photosynthesis, flavonoid and phenol content in this plant increased, which led to increased antioxidant activity of the plant (Tvrda et al., 2019). The antioxidant activity in thyme is attributed both to its extract and soluble phenolic fractions. Results of this study showed that the extract from thyme had higher antioxidant activity under various bio-fertilizers treatments, which there is maximum amount of phenolic compounds. The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play an important role in neutralizing ROS, such as free radicals, singlet and triplet oxygen and peroxides (Zahedifar et al., 2019). The most antioxidant activity was exhibited by the extract from the plants under the bio-fertilizers. Probably, bio-fertilizers could regulate the activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stresses (Salama et al., 2015). In order to counteract the oxidative stress created in plants, the high performance antioxidant defense system in plants can neutralize free radicals in plants (Zohrehvand et al., 2017). It contains antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, phenol peroxidase, and a non-enzymatic antioxidant system including ascorbate, carotenoids, phenolic compounds and proline (Sun et al., 2015). Plants with higher carotenoid content are more successful in protecting against reactive oxygen species and are better tolerated under water scarcity (Wasli et al., 2018). The correlation between different

parameters in *Thymus daenensis Celak* is shown in Table 3. Most of properties were in significant at 1% level.

Table 3: Correlation between different parameters in *Thymus daenensis Celak*

	Total dry weight per plant (g)	Essential oil yield per plant (g)	Leaf dry weight per hectare (kg)	Total Phenol	Scavenging Superoxide (%)	Scavenging Nitric Oxide (%)
Total dry weight per plant (g)	-					
Essential oil yield per plant (g)	0.80**	-				
Leaf dry weight per hectare (kg)	0.45 ns	0.77**	-			
Total Phenol	0.79**	0.91**	0.55*	-		
Scavenging Superoxide (%)	0.44 ns	0.60**	0.25 ns	0.55*	-	
Scavenging Nitric Oxide (%)	0.54*	0.74**	0.67**	0.85**	0.30 ns	-

* and **, significant difference at 5 and 1%, respectively.

The effect of fertilizer treatment was significant on leaf dry weight per hectare ($p < 0.01$) (Figure 5). The results indicated that the biofertilizer produced the maximum leaf dry weight (Figure 5). Meena et al. (2019) indicated that morphological characteristics of leaf could be changed with soil physical characteristics, soil nitrogen and climatic conditions, therefore, the optimal amount of fertilizers, especially nitrogen, could significantly improve plant growth.

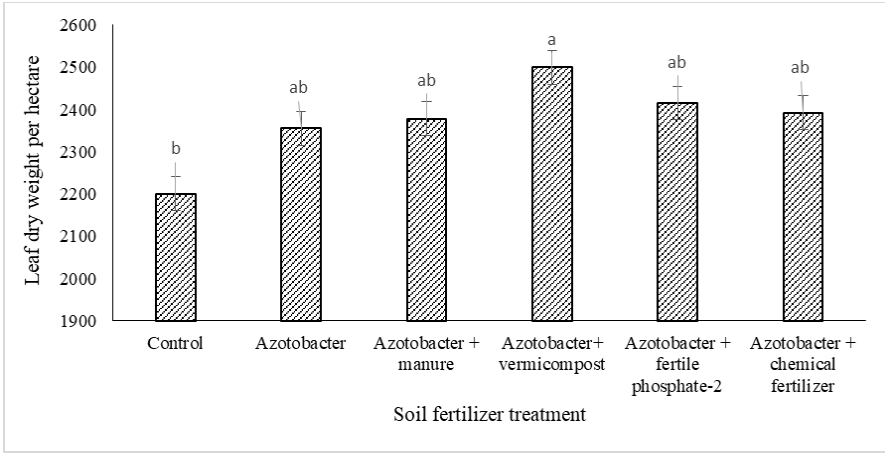

Figure 5. Effect of various biofertilizers on leaf dry weight of thyme

The results indicated that simple effects of biofertilizer treatments were not significant on leaf characteristics. Most of the plants responded to growth parameters by increasing the proportion of photosynthetic materials, which promoted the better root growth (Meena et al., 2019). A higher leaf area warrants more water availability for plants under various conditions (Salama et al., 2015). It seems that the morphological characteristics of the leaf changed with soil physical conditions, soil nitrogen, and climate. Therefore, the optimal amount of fertilizers, especially nitrogen, could be critical in plant growth and development. Some microorganisms are crucial for soil fertility by the role they play in biological fixation of nitrogen and the conversion of some nutrients from unavailable to available form (Fattahi et al., 2019). The content of some macro and micro nutrients is shown in Table 4.

Table 4: Macronutrient and micronutrient content in leaf tissue of *Thymus daenesis Celak*

Treatments	N	P	K	Cu	Fe	Zn
		(%)			ppm	
Control	1.49	0.20	1.78	9.22	160.97	18.77
Azotobacter	1.54	0.21	1.83	10.36	175.79	19.44
Azotobacter+manure	1.56	0.22	1.82	10.83	179.27	19.99
Azotobacter+vermicompost	1.58	0.24	1.89	11.56	181.11	23.98
Azotobacter+Fertile phosphate-2	1.58	0.24	1.86	11.66	179.91	21.23
Azotobacter+Chemical fertilizer	1.53	0.21	1.77	11.29	177.91	20.98

Acording to the results Azotobacter+ vermicompost and Azotobacter+ fertile phosphate-2 treatments showed the highest macro and micro nutrients content. Azotobacter and their symbiosis with plants have various effects on the improvement of plant growth and development so that they can change plant water relations and enhance the drought resistance or tolerance of the host plant (Fattahi et al., 2019). Azotobacter influence the absorption of nutrients like phosphorous and nitrogen and water uptake under stressful conditions and the synthesis of plant hormones, alleviate the impacts of environmental stresses, improve resistance to plant pathogens, mitigate root damages, affect soil aggregation, intensify the biological fixation of nitrogen and improve quantitative traits (Szpyrka et al., 2019). Known as an aerobic and physiological diazotroph, Azotobacter fixes air nitrogen and makes a balance in the uptake of macro and microelements by the plant and, in addition, it synthesizes growth stimulators, such as growth regulating hormones like auxin, different amino acids and, etc and thereby it improves the growth and development plant roots and shoots, protects plant roots against soil-borne pathogens and increases high-quality

yield per ha (Barouchas et al., 2019). It is unlikely to accomplish the goals of sustainable and organic agriculture without paying a serious attention to soil biodiversity.

Phenolic compounds are a main diverse group of plant secondary metabolites that have been linked to numerous ecological functions. The effect of different biofertilizers on total phenolic content of thyme is shown in Figure 6. The differences among the various species of a genus for TPC were also found in other medicinal plants (Majdoub et al., 2017).

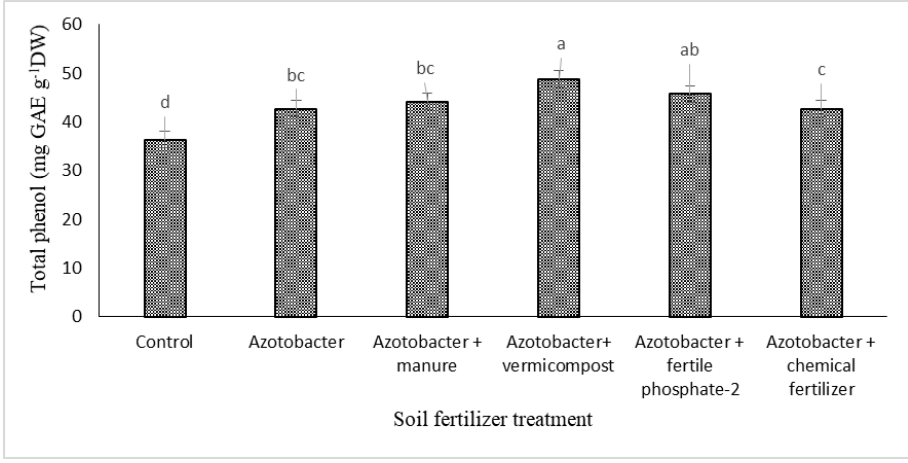

Figure.6. Effect of various biofertilizers on TPC of thyme

Comparing of our results with other studies showed two times higher amounts of TPC in thyme species than Turkish species (Manzoor et al., 2018). Environmental factors (such as soil composition, temperature, rainfall, and ultraviolet radiation) are the most effective factors on the phenolic content (Baghaie et al., 2019). The low temperatures, high radiation, pathogen infection, herbivores, and nutrient deficiency can

increase producing free radicals and reactive oxygen species (ROS) and as a result lead to increased accumulation of antioxidants such as phenolic compounds in plants (Ghasemi Pirbalouti al., 2013). In recent years, free radicals have been proven to be the most important food oxidizing agents so that in addition to their adverse organoleptic effects, they eliminate toxins and nutrients by eliminating essential vitamins and fatty acids (Lajayer et al., 2019). It is well known that phenylalanine ammonialyase (PAL) is an important marker for environmental stresses in different plant species also it plays a key role in the phenylpropanoid pathway. The differences among the various species of a genus for TPC were also found in other medicinal plants (Padash et al., 2019). Flavonoids are an important group of plant bioactive molecules occurring virtually in all plant parts. They are responsible for pigmentation and aroma in flowers also protects plants against UV damage.

CONCLUSION

The results of this study showed that biofertilizers application instead of chemical fertilizers application improved the quantitative characteristics of the thymus. It seems that the integrated fertilizer method can play an effective role in increasing the quality and quantity of thymus yield. This result could be explained by the slow release of micro and macro nutrients from the manure which increased the nutrient availability and absorption efficiency in this treatment. It could be also suggested that the higher application of biofertilizers may

increase the essential oil content, leaf dry and fresh weight, total phenol and flavonoid contents, and various radical scavenging activities.

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CHAPTER 10

EFFECT OF WEED CONTROL TIME ON YIELD, YIELD COMPONENTS AND MORPHOLOGICAL TRAITS IN *Lallemantia iberica* L.

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INTRODUCTION

In recent years, a trend in agricultural production systems has changed towards achieving high productivity and promotes sustainability over time. *Lallemantia iberica* seed contains up to 30% of a drying oil. *Lallemantia iberica* seed has traditional uses as reconstitute, stimulant, diuretic and expectorant. Farmers are developing different crop production systems to increase productivity and sustainability since ancient times (Petropoulos et al., 2020). *Lallemantia iberica* is used traditionally as stimulant, diuretic, expectorant, in the treatment of common cold, coughing, stomach and abdominal pain. It produced many secondary metabolites such as phenolic acids, flavonoids, tannins, triterpen, mucilage and oil (Tripathy et al., 2015). It possessed many pharmacological effects included analgesic, antibacterial and antioxidant effects. The current review discussed the chemical constituents and pharmacological effects of *Lallemantia iberica*. This includes crop rotation, relay cropping and intercropping of major crops with other crops. Intercropping, the agricultural practice of cultivating two or more crops in the same space at the same time is an old and commonly used cropping practice which aims to match efficiently crop demands to the available growth resources and labor (Młodzińska, 2009). The most common advantage of intercropping is the production of greater yield on a given piece of land by making more efficient use of the available growth resources using a mixture of crops of different rooting ability, canopy structure, height, and nutrient requirements based on the complementary utilization of growth resources by the

crops (Hassannejad et al., 2013). Intercrops often reduce pest incidence and improve forage quality by increasing crude protein yield of forage. These include risk of crop loss due to adverse environmental conditions, need for balanced diet, and the desire to optimize the use of labour and to optimize the use of land. The advantage is often expressed as a land equivalent ratio (LER). LER greater than one indicates that more sole cropped land than intercropped is required to produce a given amount of product (Młodzińska, 2009).

Lallemantia iberica is a very sensitive crop to weed competition, which generally results in heavy yield loss. The reduction in grain yield may vary from 23% to 87% depending on the weed species and their densities in various countries (Ahmad et al., 2019). Weeds mainly compete with crop for nutrients, soil moisture, and sunlight by covering over crop and space. Severity of yield loss depends upon weed infestation, duration of infestation as well as climatic conditions which affect weed and crop growth. Weeds can remove plant nutrients from soil more efficiently than crops (Petropoulos et al., 2020). Therefore, weeds are of crucial importance since effective and proper weed control time will result in higher seed yields of chickpea. Delayed weeding until late stages could result in irreversible damage due to weed competition. *Lallemantia iberica* is an annual herb that belongs to Lamiaceae family and spreads in southwestern Asia and Europe (Ursu & Borcean, 2012). It grows well in arid zones and requires a light well -drained soil (Ion et al., 2011). Dragon's head is a valuable species, i.e. all plant parts (leaves or seeds) can be economically used (Hassannejad & Navid,

2013). However, it is mainly cultivated for its seeds that contain about 30% oil with iodic index between 163 and 203. These seeds are used traditionally as stimulant, diuretic and expectorant as well as in food (Keshavarzi & Mosaferi, 2019). Due to the lack of relevant information, the present research was conducted to determine the effects of weed control time on yield and yield components and morphological traits of *Lallemantia iberica*.

1. MATERIAL AND METHOD

1.1. Site Description and Experimental Design

The field experiment was conducted in 2020 at the Research Farm of the Urmia University, Iran (latitude 38°05_N, longitude 46°17_E, altitude 1360 m above sea level). The climate of research area is characterized by mean annual precipitation of 285 mm, mean annual temperature of 10° C, mean annual maximum temperature of 16.6° C and mean annual minimum temperature of 4.2° C. The experimental plots were each 4 × 4 m^2 composed of the plant sowing rows as ridge with inter-row spacing of 50 cm and inter-plant spacing of 20 cm. After preparation, the plots were manually sown by wet planting on rows on April, 2020.The distance between planting rows was 30 cm. Irrigation was done twice a week according to the weather conditions and the plant need. Four time of mechanical weeds control levels were; a1, a2, a3, a4, a5, and a6: The third true leaf, the sixth true leaf, the first flowering branch, the third flowering branch, flowering, and seeding times respectively. The experiment was arranged in a randomized

complete block design, with three replications. The mean annual rainfall and temperature were shown in Figure 1.

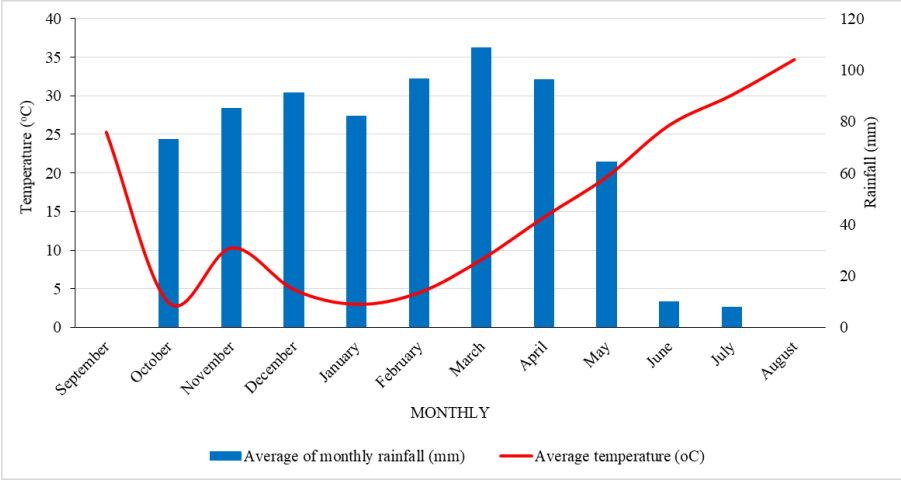


Figure 1. Climatic data of the experiment city (Rahimi et al., 2019)

1.2. Measurement of Traits

To specify plant heights, number of capsule per plant, number of seeds per plant, and lateral stem, biological yield, seed yield per ha and harvest index were selected from the middle of the plots and then, they were measured. In order to determine the biological yield an area equal to 1 m^2 was harvested from middle part of each plot considering marginal effect.

1.3. Total Phenolic Content (TPC)

The total phenol content of the extracts was determined using Folin-Ciocalteu and Hurwitz (1984) method with slight modification.

According to this method, 1 ml of Folin-Ciocalteu (diluted 1:10) was added to 50 ml of the plant extract. Then the solution was mixed with 1 ml of sodium carbonate (10%) and they were incubated at room temperature and dark for 60 minutes. Finally, the absorbance of the solution was measured using a spectrophotometer at 750 nm. Total phenolic content was expressed in mg / kg of gallic acid in 100 g of extract using standard gallic acid curve.

1.4. Total Flavonoid Content (TFC)

In order to determine the content of flavonoid in the extracts, 50 ml of the extract was mixed with 1 ml of distilled water in the test tube and then 0.075 ml of sodium nitrite (5%) was added and after 5 min 15 min. 0.5 ml of AlCl3 solution (10%) was added and after 0.5 minutes 0.5 ml NaOH (1 M) was added and the final volume of the solution was distilled to 3 ml. The intensity of pink color emerging in solution at 510 nm was read by spectrophotometer, total flavonoid content was expressed in milligrams of quercetin equivalents in 100 g of extract using standard quercetin curve.

1.5. Essential Oil

Essential oil extraction was performed using Clevenger apparatus (distilled water). Then, 10 g of dried leaves were poured into a 1000 ml balloon, and about 100 ml of distilled water was added and extraction was performed. The extraction time was about 3 hours. During this time, the volatile compounds were extracted with water vapor and after

cooling, a distinct layer on the surface of the water was visible in the graduated tube of the Clevenger machine (Adams, 2007).

1.6. Mucilage Yield

To measure the mucilage, boil one gram of dry seed in 10 ml of 0.1 normal hydrochloric acid until the color of the seed coat changes, and after observing this situation, the initial mucilage solution is obtained. Another container was transferred. Then the remaining seeds were washed twice in the first container and each time with 5 ml of boiling water and added to the mucilage solution. 60 ml of 0.96 ethyl alcohol was added to the obtained mucilage solution and kept in the refrigerator for 5 hours. Mucilage analysis was performed with an accuracy of 0.001 (Alves et al., 2016). The mucilage yield per unit area, which is a function of the mucilage percentage and grain yield, was calculated by the following equation (Tripathy et al., 2015):

Performance mucilage = mucilage percentage x seed yield Eq. (1)

1.7. Radical Scavenging Activity

The amount of DPPH (2,2-diphenyl-1-picrylhydrazyl) stable radical scavenging was determined with little change by Tripathy et al (2015). 40 µl of the extract was mixed with 2 ml of DPPH methanol solution (0.004%). The adsorption of the mixture was read after 30 min incubation (at room temperature and dark) at 517 nm.

$$\text{Inhibition } (\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \qquad \text{Eq (2)}$$

Where A control and A sample are the absorbance of the control and the sample respectively.

1.8. Super Oxide Radical Scavenging Activity

To measure superoxide anion radicals, superoxide anion radicals were generated by a pyrogallol autoxidation system. The test tube containing 9 ml of Tris buffered saline (pH = 8.2, 50 mmol / l) was incubated for 20 minutes in a mortar at 25 ° C. 40 microliters of pyrogallol solution (45 mmol / l pyrogallol in 10 mmol hydrochloric acid), previously incubated at 25 ° C, was injected into the upper part of the test tube using a microliter syringe. And it was mixed. The mixture was incubated at 25 ° C for 3 minutes and then 1 drop of ascorbic acid (0.035%) was immediately inoculated to complete the reaction. The adsorption of the mixture at 420 nm was recorded as A0 after 5 min, and this A0 shows the rate of pyrogallol autoxidation. The A1 autoxidation rate was increased by the same method only with a certain amount of extract (10 μL) in Tris buffer. At the same time, a control blank of reactive materials was considered as A2. The percentage of radical accumulation was calculated using the following formula (Bose et al., 2019):

Super oxide radical scavenging (%) = [(A0-A1/A0)] ×100 Eq. (3)

Where A0 is theabsorbance of the Tris-HCl buffer with pyrogallol, A1is the absorbance of the extract addition.

1.9. Nitric Oxide Radical Scavenging Activity

Nitric oxide radical inhibition was calculated using Griess Illosvoy reaction. In this method, the Griess Illosvoy reaction agent was modified by substituting naphthylene diethylamide dihydrochloride (0.1% volume / weight) instead of 1-naphthylamine (5%). 3 ml of the reaction solution was incubated with 2 ml of sodium nitroprusside (10 mM), 0.5 ml of saline phosphate buffer, and 40 ml of the plant extract for 25 minutes at 25 ° C. After incubation, 0.5 ml of the resulting solution was mixed with 1 ml of sulfanilic acid (0.33% in 10% glacial acetic acid) and allowed to stand for 5 min to complete permanent denaturation. Then 1 ml of naphthylethylenediamine dihydrochloride was added to the mixture and allowed to stand for 30 minutes at 25 ° C. A diffuse pink color appeared in the light background. The absorbance of this solution was read at 540 nm against a blank. The percentage of nitric oxide radical accumulation was calculated using the following formula (Bose et al., 2019):

$$\text{Nitric oxide radical inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad \text{Eq. (4)}$$

Where A control is absorbance of control sample and A sample absorbance in the presence of the samples of extracts or standards.

1.9. Statistical Analysis

Statistical analysis of the data was performed with MSTAT -C software. Duncan multiple range test was applied to compare means of each trait at 5% probability.

2. RESULT AND DISCUSSION

2.1. Plant parameters

Statistical analysis of the data indicated that different intercropping patterns and weed management practices had significant effect on plant height of *Lallemantia iberica* (Table 1). Maximum plant height (92 cm) was obtained in the third true leaf of *Lallemantia iberica* (a_1). Minimum plant height was recorded in the a_6 treatment (Figure 1).

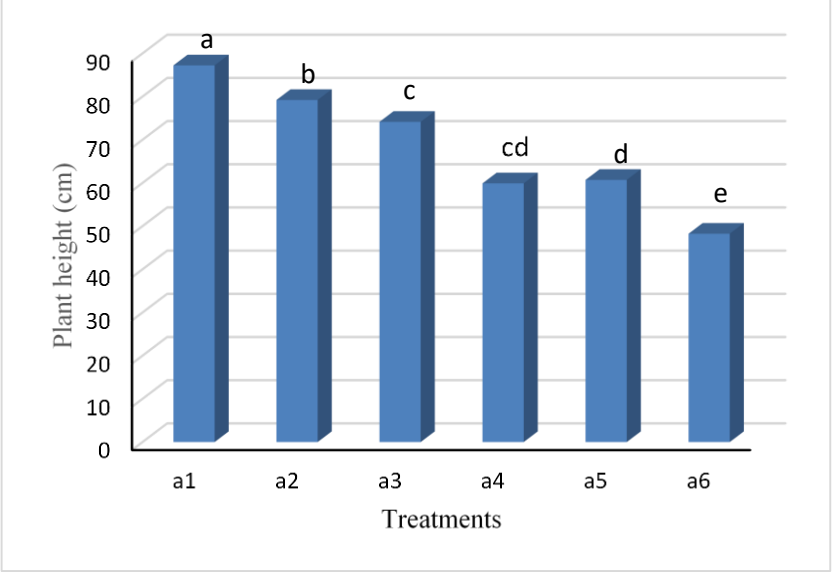


Figure.1. Plant height (cm) as affected by various weed time

However, this value was not significantly different from the mean plant height recorded under a_2-a_6 treatments. The canopy characteristics of crops are not constant, but may change due to the presence of other crops species (Nasrollahzadeh et al., 2014). This result is similar with finding of Tripathy et al (2015) who reported that plant height of maize intercropped with both beans and pumpkin were adversely affected by intercropping conditions. Maize plants were taller for sole crops compared to when intercropped with beans, both in the presence of weed infestation. In other results, (Nazemi et al., 2012) did not find any significant difference in plant height between mono cropping and intercropping of maize with sugar bean and ground nuts. According to Alves et al (2016), on average, maize and beans on unwedded plots were 17% taller than those in weeded plots due to competition for light between crops and weeds.

Table 1: Analysis of variance of selected parameters of *Lallemantia iberica*

	Mean square						
SOV	Plant height (cm)	Lateral steam (cm)	Number of capsule per plant	Number of seeds per plant	Biological yield (ha)	Seed yield per ha	Harvest Index
Block	1.32	1.15	0.35	0.54	0.79	0.25	0.12
Treatment	10.25**	0.58ns	2.01ns	3.68ns	18.26**	4.05ns	1.25ns
Error	2.56	5.69	6.87	8.66	0.11	7.13	5.25
CV (%)	5.32	6.25	10.25	4.36	3.69	4.69	7.12

**: Significant at 1% probability level. ns: not significant.

Due to significant relationship for plant height and biological yield in various weed control time, these parameters were shown in Figures 1 and 2, respectively. As can be seen, the plant height was decreased order, while, the increase order was observed in biological yield per hectare (Figures 2).

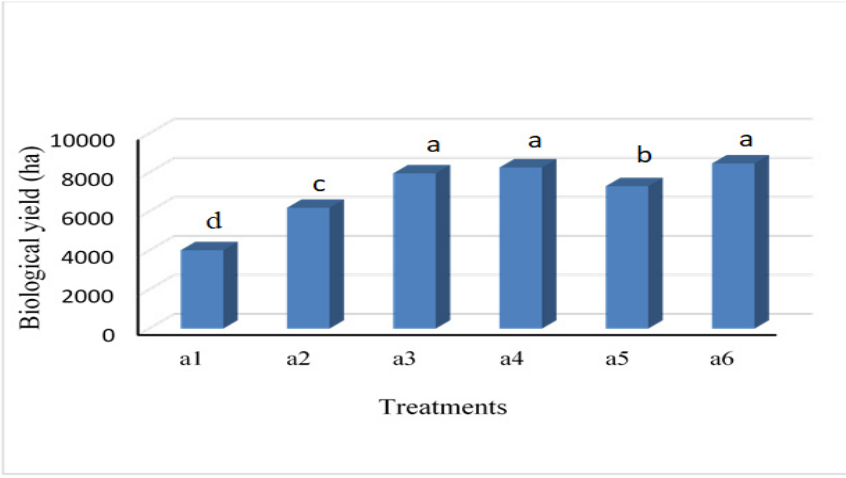


Figure 2. Biological yield (ha) as affected by various weed time

2.2. Total Phenolic (TPC) and Flavonoid Contents (TFC)

Total seed phenol and flavonoid contents as affected by various weed times shown in Figure 3. Generally, there was no significant between treatments in total phenolic and flavonoid contents as affected by various treatments. Phenolic compounds are a main diverse group of plant secondary metabolites that have been linked to numerous ecological functions. The differences among the various species of a genus for TPC were also found in other medicinal plants (Kalvanagh and Heris, 2013). Comparing of our results with other studies showed two times higher amounts of TPC in thyme species than Turkish species

(Alves et al., 2016). Environmental factors (such as soil composition, temperature, rainfall, and ultraviolet radiation) are the most effective factors on the phenolic content (Khan et al., 2016).

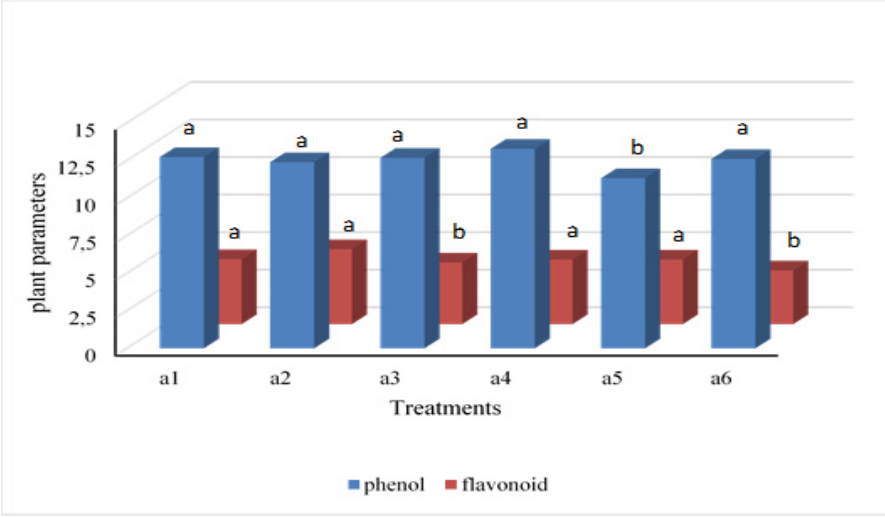


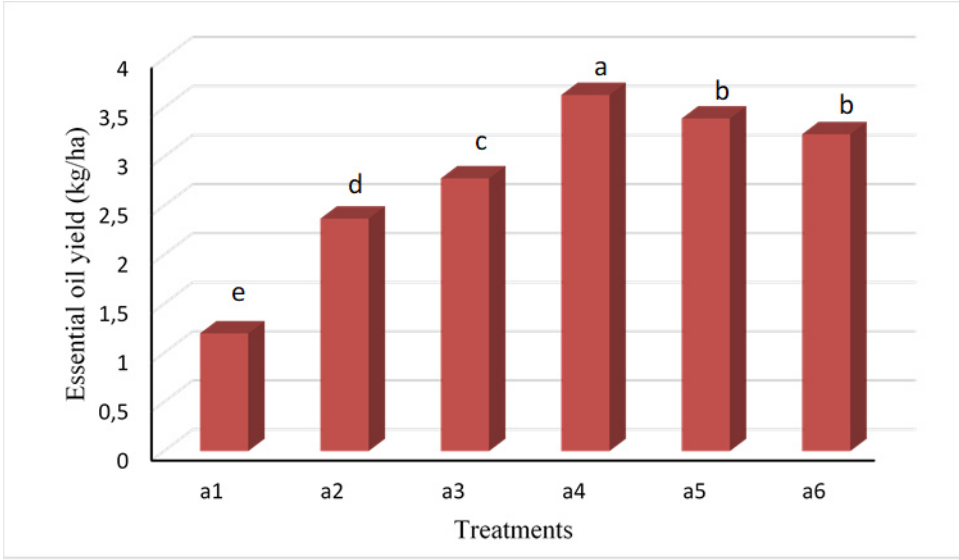
Figure 3. Total phenol and flavonoid contents as affected by various weed times

The low temperatures, high radiation, pathogen infection, herbivores, and nutrient deficiency can increase producing free radicals and reactive oxygen species (ROS) and as a result lead to increased accumulation of antioxidants such as phenolic compounds in plants (Alves et al., 2016). In recent years, free radicals have been proven to be the most important food oxidizing agents so that in addition to their adverse organoleptic effects, they eliminate toxins and nutrients by eliminating essential vitamins and fatty acids (Sivanesan et al., 2016). It is well known that phenylalanine ammonialyase (PAL) is an important marker for environmental stresses in different plant species also it plays a key role in the phenylpropanoid pathway. The differences

among the various species of a genus for TPC were also found in other medicinal plants (Nazemi et al., 2012). Flavonoids are an important group of plant bioactive molecules occurring virtually in all plant parts. They are responsible for pigmentation and aroma in flowers also protects plants against UV damage. Therefore UV radiation increases strongly flavonoid synthesis (Bose et al., 2019). There were significant differences among the studied species for TFC. Variation in TFC may be explained based on of difference in the genetic background of mullein species.

2.3. Essential Oil and Mucilage Yield

Essential oil yield, and mucilage yield as affected by various weed times shown in Figure 4. The significantly lower content of essential oil yield was obtained under various treatment. The highest mucilage yield was observed in a_4 and 4_5 treatments respectively. Several number of studies have demonstrated that the chemical composition of essential oils varies with geographical location, growing region, soil type, climate, altitude from sea level, and water availability. Even season, e.g., before or after flowering and the hour at which setting is done, affects the chemical composition of essential oils (Fokina et al., 2018). Our results are consistent with Zargari who reported that the quantity and quality of *L. iberica* essential oils were influenced by genotype, but climatic conditions and the interactive effect of plant and



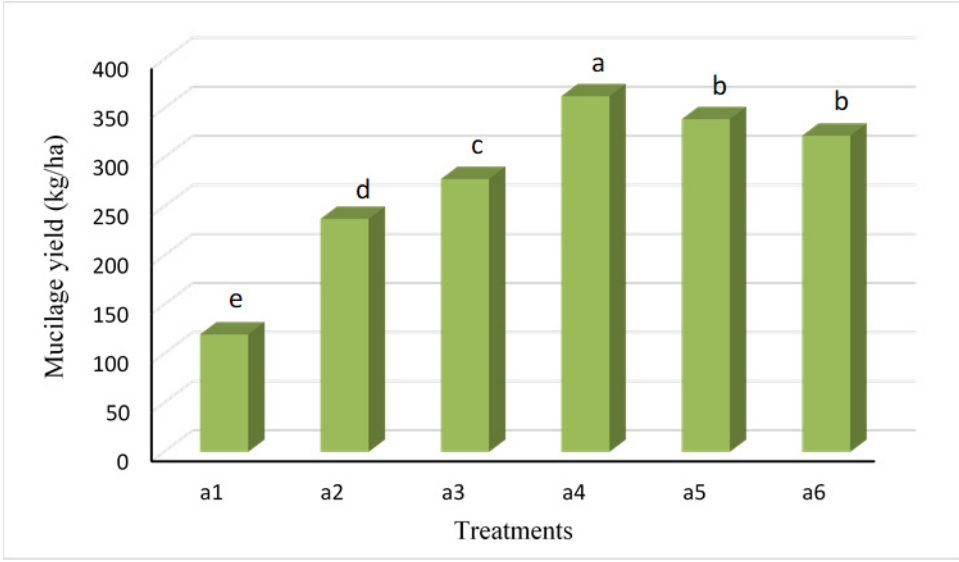


Fig.4. Essential oil yield and mucilage yield as affected by various weed times

Furthermore, plants had more of a chance for organic matter accumulation in the first sowing date compared to the second. The results obtained for the effect of weed control time on *L. iberica* and the production of more essential oil are in agreement with that previously reported about chamomile, *Dracocephalum moldavica* L., and fennel (Kalvanagh et al., 2013). Previous studies have indicated that Phylum and *L. iberica* weed in the six true leaf have a longer growth period than those weed in the three true leaf time, so they are in a better place to synthesize seed components, especially mucilage (Zargari, 1998). The mucilage percentage of *L. iberica* was increased by increasing of weed control time (Khan et al., 2016). In a study on Nazemi et al (2012) reported that the early weed of *L. iberica* enhanced mucilage yield significant compared to the control. Likewise, some researchers have attributed the higher seed and mucilage yield of phylum to the late weed control time. Thus, it was shown that the higher mucilage yield was associated with the higher seed yield and mucilage percentage under the influence of optimal environmental conditions. Modern pharmacological and toxicological studies have demonstrated that crude extracts of the seeds and some of its active constituents might have protective effect against nephrotoxicity and hepatotoxicity induced by either disease or chemicals (Gholamnezhad et al., 2016). Very interesting is the isolated oil of the oilseed crop of *Lallemantia*, better known as lberian dragonhead, showing a very high content of linoleic acid exceeding that of linseed oil, and showed high theoretical iodine values. Unsaturation in the oils were used to introduce epoxides environmental conditions also influenced this trait (Alves et al., 2016).

by epoxidation with in situ generated proxy acetic acid (Ghannadi et al., 2015). Nowadays herbal science has advanced and medicinal plants along with chemical drugs are used to treat some diseases (Sivanesan et al., 2016). During the past decade the use of complementary medicines, such as herbal medicinal substances in dementia therapy, has been studied (Bose et al., 2019) based on traditional medicine, which has been practiced in many parts of the world. The knowledge of these important sources could profitably apply to allopathic science (Khan et al., 2016). Knowledge of the phytochemical properties of medicinal plants is essential to improve their medicinal effect and facilitate the design of harvesting, processing, and storing of the seed. Various types of cleaning, grading and separation equipment may be designed on the basis of the physical properties of the seed.

2.4. Radical Scavenging Activity

Different radical scavenging activities as affected by various weed times is shown on Figure 5. Significant differences were obtained among various treatments. Several studies have revealed that early weed control time improve this trait compared to the control, and the integrated treatments were more effective than the simple treatments, which can be attributed to the positive effect of environmental conditions. Research also shows that there is a direct relationship between the weed control time, content of phenol compounds and antioxidant activity (Etratkhah et al., 2019). The higher content of phenol compounds as a free radical scavenger is the main reason for the higher antioxidant activity of the plant extracts. Research has shown

that early control weed time had more beneficial effects than other times. Oxygen radicals are capable of destroying cell membrane lipids, proteins, and hereditary substances (Jalilehvandi et al., 2017). It is well known today that oxidative degradation caused by the activity of these molecules causes and promotes a number of chronic diseases such as cardiovascular disease, cancer disease (Ghannadi et al., 2015). Antioxidant compounds are needed to counteract the toxic effect of oxygen free radicals. Plant cells usually use enzymatic antioxidant systems such as super oxidase dismutase, catalase, antioxidant metabolites, phenol, etc. to solve this problem (Carrier et al., 2003; Kim et al., 2009). Oxidative stress is caused by the overproduction of free radicals and reactive oxygen species and the weakening of the antioxidant system due to the low production of endogenous antioxidants (Ram et al., 2005).

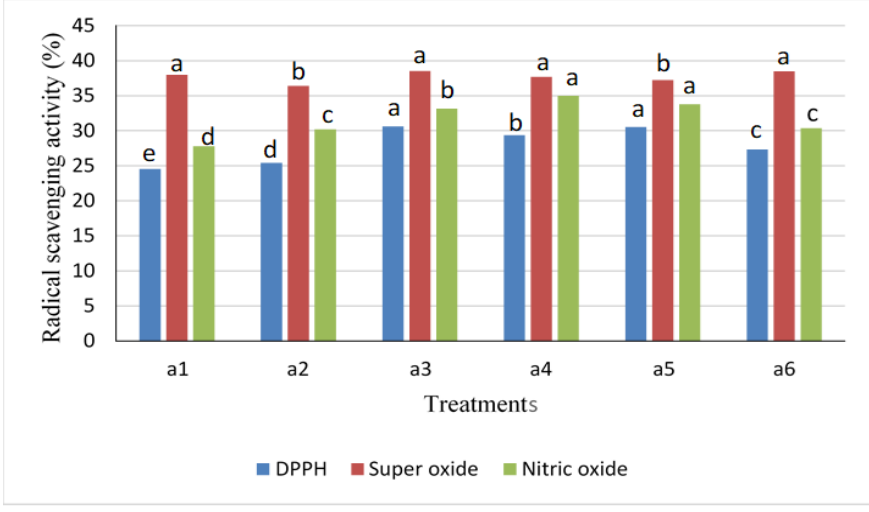


Figure 5. Different radical scavenging activities as affected by various weed times

CONCLUSION

The results showed that by prolonging weed-infested period, biological yield, total phenol and flavonoid contents were increased, but by increasing weed-infested period, plant height was decreased. At weed infested all period, of *Lallemantia iberica L.* growing season, superoxide radical scavenging activity had the highest and DPPH radical scavenging activity compared with other weed species.

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CHAPTER 11

ESSENTIAL OIL COMPOSITION IN DIFFERENT PLANT PARTS OF *Scorzonera acuminata*

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INTRODUCTION

Turkey is one of the industrializing countries and one of the important gene centers of plant diversity in the world. Collected data revealed, that the Anatolian peninsula displays the richest flora compared with Southwest Asia, the Mediterranean basin and whole Europe. The number of flowering plant taxa in Turkey is estimated as about 10.000, near to the number of whole Europe (Davis, 1965-1985; Davis et al., 1988; Guner et al., 2000). These taxa are distributed in different phytogeographical regions and include nearly 3.300 endemics, which are mostly found in the Irano-Turanian region (Ozgokce & Çelik, 2004; Simsek et al., 2004). The number of plant species used in Turkey as folk remedies was previously estimated at around 500, but recently this number has been figured around 1.500 Baytop (1999). Although scarcely practiced in Turkey, the traditional Greco-Arabic (Unani) medicine is still being practiced widely in the south and southeast regions of the country.

The *Scorzonera* L. (Asteraceae) genus contains about 160 species belonging to the subtribe Scorzonerinae Dumort. of the tribe Cichorieae, can be found in the more arid regions of Eurasia and northern Africa (Brehmer & Anderberg, 1994; Nazarova, 1997). The genus is represented by 52 (59 taxa) species, 31 of them endemic to Turkey (Coskubcelebi et al., 2015). Many members of this genus, such as *S. hispanica* L. (Zidorn et al., 2000), *S. humilis* L. and *S. cretica* (Willd.) (Zidorn et al., 2000, 2003). , *S. mongolica* Maxim. and *S.*

austriaca Willd. (Zhu et al., 2009)*, S. pseudodivaricata* Lipsch. and *S. radiata* Fisch. (Tseveguren et al., 2006; Wang et al., 2009) were used in Anatolian folk treatment. One of the endemic taxon of the genus is *Scorzonera acuminata* Boiss., which distributed mainly in Central Anatolia. They are distinctly caulescent plants with characterized subcoriaceous acuminate leaf end glabrous achene and growing in calcareous rocky places of the inner Anatolia (Coskuncelebi et al., 2015). According to the Red Data Book of Turkish Plants [15] and latest evalutions performed by (Ekim et al., 2000) revealed that its IUCN threatened categories are LC (Low Critical).

In the development of human culture and human civilization plants have already played and are playing an remarkable role up to day. If we look at medicine applications of different civilizations, plants with medicinal value are coming every time to the forefront. As a wide acceptation such plants can be determined as main sources of traditional medicinal applications and we know that nowadays modern medicines make use of them. Dar et al. (2017) stated, that medicinal plants have been used to heal health disorders, to increase flavor of food and to conserve it. Further, such plants had wide use in preventing diseases epidemics. Addtionally, Hassan (2012) explained, that plants with medicinal value forms a huge group of plants concerning great interest, because of its pharmaceutical, cosmetic and nutritional values.

Bioactive compounds synthesized in plants with medicinal value may vary greatly depending on a number of internal and external factors such as plant health and age, used plant part, growth stage and

harvesting time (Figueiredo et al. 2008; Telci et al., 2009). The highest essential oil, for example, is present in leaves of certain plants, but in flowers of others. On the other hand, soil and climatic conditions, production practices and postharvest operations play positive or negative effects on the amount and quality of bioactive compounds as well (Figueiredo et al., 2008).

Up to our knowledge the essential oil composition of *S. acuminata* is not investigated. Some investigations were made in related species like *S. undulata* spp. *Deliciosa* (Harkati et al., 2012), *S. undulata (*Boussada et al., 2008), *S. sandrasica* (Ugur et al., 2010) and *S. calyculata* (Ayromlou et al., 2019). The present study presents the findings about the esssential oil composition of *S. acuminata* plant parts to reveal the potential value of this species.

1. MATERIAL AND METHOD

1.1. Plant Material

S. acuminata was collected in Ankara, Elmadağ, Gurlevik walley (A4), calcareous rucky places and meadow fields (at heights of ~900 m and 1000 m) in the inner part of Turkey. Voucher specimens (no. Makbul 215 & Coşkunçelebi; Figure. 1) was deposited in the Herbarium of the Department of Biology, Recep Tayyip Erdogan University (RUB) and Herbarium of the Department of Biology, Karadeniz Technical University (KTUB), Turkey. The plant materials was identified immediately after collection (Coskuncelebi et al., 2015; Chamberlain (1975) and air-dried at +4 ^{o}C temperature for later analysis.

Figure 1. Collected *Scorzonera acuminata* plant

Plants samples were seperated to their root, stem, leaf and seed for essential oil analysis.

1.2. SPME Analysis

For HS–SPME a SPME device (Shimadzu, Japan was used. The plant materials (1.00 g, each) were powdered and placed in a 10 mL vial sealed with a silicone-rubber septum cap. The fiber was pre-conditioned according to the manufacturer instructions. At equilibrium, the fiber was exposed to the headspace for 1 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of GC or GC–MS system. For GC a Shimadzu GC-MS-QP 2010 equipped with equipped with a CP 5MS (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was

programmed from 40°C to 240°C at 2°C/min, then isothermal at 220 °C for 20 min. Helium was used as a carrier gas with a constant flow at 1 mL/min. The temperature of injector and detector was 240°C. The determination of essential oil components was done using the Wiley, Nist Mass Spectral and aroma method databases.

1.3. Data Analysis

Obtained chemical data was analysed using one-way analysis of variance (ANOVA). Each species was analyzed statistically to show existing differences regarding constitutents at different developmental stages. Determined significant differences among mean values were tested using Duncan Multiple Range Test ($P < 0.01$). $x' = \sqrt{x} + 1$ transformation was applied to mean values of quercetin and rutin contents in the investigated, because these compounds were not detected in some cases.

Biplot, Principal Component Analysis (PCA) and Cluster Analysis were performed by using XLSTAT 2021 Statistical Program to analyze the relevance between plant ontogeny and chemical content/composition of investigated species. Scatter plot diagrams were created by utilizing the obtained data (Maione & Barbosa, 2019). Biplots and Cluster diagrams were developed to differentiate investigated material based on HPLC and GC-MS analysis. separately for both species.

2. RESULTS AND DISCUSSION

The essential oil composition of different plant parts of *S. acuminata* are given in Table 1. A number of total 66 different essential oil components could be detected in different plant parts of this species. It is obvious, that different plant parts of this species differ in their essential oil composition. For example, some essential oil components could be detected only in root, only in stem, only in leaf and only in seed or differed according to plant parts of *S. acuminata* and their pecentage also varied.

The 15 essential oil components α-Cubebene, α–Gurjunene, Farnesene, α-Himachelene, α-Curcumene, α-Muurolene, Carotol, α-Acerenol α-Bisabolol, Juniper Camphor, Nonenal, Apiole, Furan, α-Ionone and Undecalactone could be detected only in roots of this species. The components only detected in stem parts, a number of three, were Farnesal, Heptadecyl alcohol and Dodecalactone. If we look at the leaf parts of this species, Limonene, α-Humulene, Cedrol, α-Sinensal, Caprylaldehyde, Heptyl methy ketone, Myristic acid and Hedione. Further, 3 components, namely Tridecylaldehyde, Docosane, Methyl Jasmonate and Methyl Laurate were detected only seeds in seeds of *S. acuminata*.

α-Copaene, β-Caryophyllene, β-Ionone, Capronaldehyde, Pelargonaldehyde, Pentadecanol, Myristic alcohol, Tetradecane, Pentadecane, Hexadecane, Heptadecane, Octadecane, Heneicosane and Phytone were detected in all plant parts of *Scorzonera acuminata*. The

highest amounts were detected with 27.16 % for Beta β Caryophyllene in root, 12.97 % for β Caryophyllene in stem, 25.96 % for β-Caryophyllene in leaf, and 22.02 % for Lauryl alcohol in seed.

The established chemical classes of essential oil composition of *S. amunicata* is given in Table 2. In fact, *S. acuminata* plant parts could be clearly differentiated based on their essentail oil composition (Table 1 and Figure 1,2). Regarding all plant parts, essential oil components could be grouped into six classses: monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, the group of alcohols, ketones, aldehydes and furans, the group of alkanes, alkenes, alkynes and arenes and the group of ethers, carboxylic acids and esters.

Table 1: Percentage of essential oil composition of *S. amunicata* plant parts

No	**Compounds**		***S. acuminata***			
	Monoterpene Hydrocarbons	***RI****	Root	Stem	Leaf	Seed
1	α- Pinene	933	1.73	-	0.45	-
2	Limonene	1028	-	-	1.26	-
3	***Sesquiterpene Hydrocarbons***					
4	α- Cubebene	1346	1.28	-	-	-
5	Cyclosativene	1367	1.62	0.85	-	-
6	α- Copaene	1375	0.76	2.69	0.49	0.15
7	α- Gurjunene	1406	9.12	-	-	-
8	β-Caryophyllene	1418	27.16	12.97	25.96	4.78
9	Farnesene	1452	2.01	-	-	-
10	α- Humulene	1458	-	-	0.9	0.19
11	α- Himachelene	1449	0.83	-	-	-

12	α- Curcumene	1480	2.81	-	-	-
13	Germacrene D	1485	-	-	0.84	0.32
14	β-Ionone	1490	0.63	0.91	3.28	0.29
15	α- Muurolene	1497	1.53	-	-	-
	Oxygenated Sesquiterpenes					
16	Caryophyllene oxide	1589	-	1.87	3.59	0.56
17	Carotol	1601	4.11	-	-	-
18	Cedrol	1615	-	-	0.85	-
19	α- Acerenol	1632	1.59	-	-	-
20	α- Bisabolol	1688	1.1	-	-	-
21	Juniper camphor	1696	2.61	-	-	-
22	α- Sinensal	1732	-	-	0.58	-
23	Farnesal	1753	-	1.39	-	-
	Alcohols, Ketones, Aldehydes, Furans					
24	Capronaldehyde	801	4.02	1.79	0.77	0.16
25	Caprylaldehyde	1003	-	-	0.49	-
26	Phenylacetaldehyde	1042	-	-	0.39	0.23
27	Pelargonaldehyde	1107	0.99	3.29	8.34	0.15
28	Heptyl methyl ketone	1108	-	-	0.62	-
29	Nonenal	1163	1.06	-	-	-
30	Capraldehyde	1206	-	-	0.46	0.23
31	Decyl alcohol	1278	1.14	1.46	-	-
32	α- İonone	1473	0.59	-	-	-
33	Lauryl alcohol	1493	-	2.83	-	22.02
34	Tridecylaldehyde	1511	-	-	-	0.13
35	Tridecanal	1573	0.75	1.51	0.74	-
36	Tridecyl alcohol	1580	1.35	3.02	-	-

37	Myristic alcohol	1680	1.28	2.83	0.63	0.22
38	Pentadecanol	1784	1.07	0.88	0.7	0.17
39	Cetyl alcohol	1881	-	0.87	1.19	0.18
40	Heptadecyl alcohol	1969	-	1.25	-	-
41	Phytol	2115	-	1.08	2.58	0.59
	Alkanes, Alkenes, Alkynes, Arenes					
42	Tetradecane	1400	2.18	1.43	0.72	0.18
43	Pentadecane	1500	1.9	2.58	0.4	0.23
44	Hexadecane	1600	10.63	9.71	3.93	0.91
45	Heptadecane	1700	1.77	4.69	2.19	0.44
46	Octadecane	1800	2.87	2.89	1.91	0.57
47	Nonadecane	1901	-	3.91	1.53	21.08
48	Eicosane	2001	-	1.01	-	2.7
49	Heneicosane	2100	2.17	10.24	18.65	40.38
50	Docosane	2201	-	-	-	0.39
	Ethers, Carboxylic Acids, Esters					
51	Furan	991	0.79	-	-	-
52	Geranyl acetone	1454	-	0.84	1.73	-
53	Methyl Laurate	1526	-	-	-	0.16
54	Citonellyl butyrate	1532	-	1.23	3.77	0.44
55	Nonanoate	1548	1.02	0.91	-	-
56	Undecalactone	1577	0.79	-	-	-
57	Methyl Jasmonate	1649	-	-	-	0.48
58	Furan-2-carboxylic	1649	2.16	2.61	0.79	-
59	Dihydrojasmonate	1657	-	2.44	-	0.19
60	Hedione	1658	-	-	0.93	-

61	Dodecalactone	1672	-	2.36	-	-
62	Apiole	1683	0.78	-	-	-
63	Myristic acid	1753	-	-	0.71	-
63	Phytone	1841	0.69	5.91	6.59	0.94
64	Hexadecenoic acid	1922	-	-	1.02	-
65	Methyl Palmitate	1925	1.12	5.73	-	0.67
Total			100.00	99.98	99.98	100.00
Number of detected compounds			37	34	35	31

Specially, the group of alkanes, alkenes, alkynes and arenes were highest in all plant parts (21.52 % in root, 36.46 % in stem, 29.33 % in leaf and 66.88 % in seed). Further, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, the group of alcohols, ketones, aldehydes and furans and the group pf ethers, carboxylic acids and esters were present in all plant parts. Sesquiterpene hydrocarbones (45.74 %) were highest in roots; the group of alkanes, alkenes, alkynes and arenes were highest in stem (36.46 %), Sesquiterpene hydrocarbones (31.47 %) in leaves and again the group of alkanes, alkenes, alkynes and arenes in seeds (66.88 %).

The chemical composition of essential oils is affected by environmentally-regulated factors (light, precipitation, growing site, and soil) and endogenous factors (anatomical and physiological characteristics of the plants). This leads to chemical variation between different parts of the plants (Barra ,2009).

66 different essential oil components could be detected in different plant parts of *Scorzonera acuminata* in the present study, whereas their proportion and distribution were different in every plant part. Research about the essential oil composition of *Scorzonera* species are rarely and in *S. acuminata* they are lacking.

Boussada et al. (2008) investigated the essential oil composition of *Scorzonera undulata* subsp. *deliciosa*. The oil of this species was characterized by a high amount of fatty acids and their esters (60.1%) and the major constituents were found to be methyl palmitate (methyl hexadecanoate) (30.4%) and methyl linolenate (23.9%). Other important chemical group consisted of aliphatic hydrocarbons in the ratio of 23.2%, among them, heneicosane (12.2%) and octadecane (4.4%) were the predominant compounds. Harkati et al. (2012) investigated the volatile compounds of *Scorzonera undulata* (Guiss) in Algeria. They detected 43 compounds, major compounds were hexadecanoic acid (42.2%), n-tetradecanoic acid (16.1%), 9-octadecenoic acid (7.7%) and 9- hexadecenoic acid (4.5%).

Scorzonera sandrasica essential oil was investigated by (Ugur et al. (2010). The main essential oil constituents of this species were caryophyllene oxide (19.7 %), manoyl oxide (16.5 %) and manool (11.3 %). Carvacrol, beta caryopyllene and, aromadendrene could be detected in lower amounts. The essential oil composition of different parts of *S. acuminata* is different from above mentioned species. Zhao et al. (2010) analyzed the constituents of essential oils from different organs

of *S. albicaulis* Bunge and identified by GC-MS a total of 40 compounds. Aliphatic acid and ester represented the two most abundant chemical classes in different organs.

PCA is a useful statistical analysis for the differentiation of plant materials and its results can give information about differences and similarities of various species regarding their chemical composition (Smelcerovicc et al., 2008; Bertoli et al., 2011). PC1 contributed 77.57 % and PC2 contributed 19.51 % to the present variation based on essential oil composition, which was very useful in the differentiation of investigated material.

In the present study, we used statistical tools to evaluate the chemical composition of *S. acuminata* plant parts. This analysis method was used to differentiate different plant parts of *S. acuminata* regarding their essential oil composition. Based on obtained data the essential oil composition of the seeds and stem of *S. acuminata* was clearly different from the root and leaf parts (Figure 2 and 3).

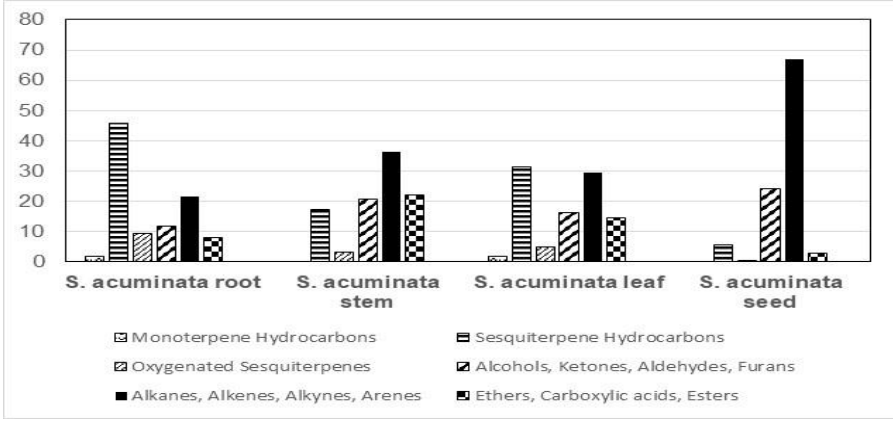

Figure 2. Differentation of *Scorzonera acuminata* plant parts based on determined chemical classes

Monoterpene hydrocarbones, sesquiterpene hydrocarbones and oxygenated sesquiterpenes were effective in this differentation. *S. acuminata* seeds differed from stem and leaf parts regarding the group of alkanes, alkenes, alkynes and arenes oxygenated sesquiterpenes.

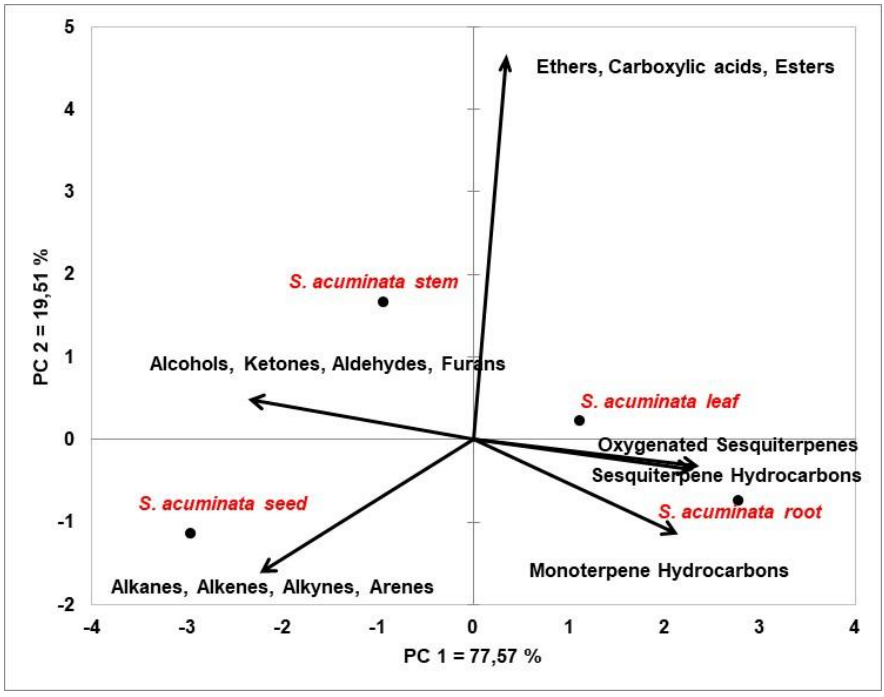

Figure 3. Biplot Analysis of *S. acuminata* plant parts based on determined chemical classes

This seperation can be seen better in the calculated dendogramme (Figure 4). In the created cluster the root and seed parts of *S. acuminata*, specially root part, were different based on determinated essential oil components.

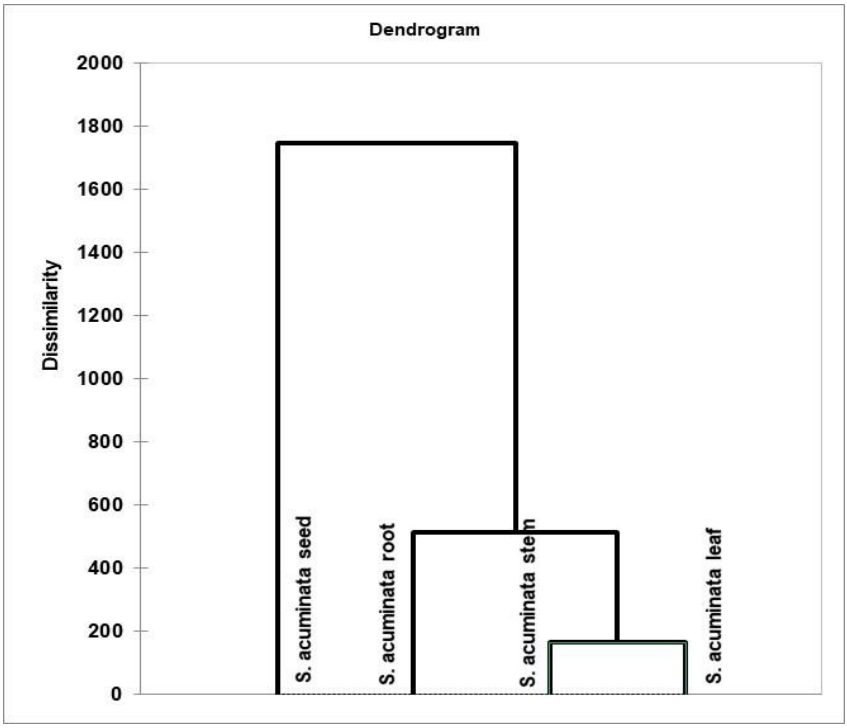

Figure 4. Dendogramme of *S. acuminata* plant parts based on esssential oil composition

PCA and additionally Cluster Analysis tools are helpful in genotype characterization and related grouping calculated on similarity basis (Mohammadi & Prasanna, 2003; Peeters & Martinelli, 1989). PCA analysis can be used in the differentiation of plant materials, further differences of various species based on their chemical composition could be achieved (Smelcerovic et al., 2008; Bertoli et al., 2011). If these two methods are combined characters which are critically contributing for genetic variability in crops can be analysed (Rachovska et al., 2003). Biplot is a further step in PCA, where

factors contributing to the differentiation of obtained variation could be grouped and detected (Aghae et al., 2010). In the present study investigated *S. acuminata* plant parts could be clearly differentiated based on their essential oil composition. Specially seed and root parts of this plant species differed based on essential oil composition from leaf and stem parts.

In conclusion, the essential oil composition of *S. acuminata* was investigated for the first time. The present results indicate differences in the essential oil composition of different plant parts of this species. Data, presented here could also be useful in determining the forthcoming goals for further wide-ranging studies on this species as well as enriching our current knowledge about *S. acuminata* chemistry.

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CHAPTER 12

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN DIFFERENT PLANT PARTS OF *Viburnum opulus* AT DIFFERENT ALTITUDES

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INTRODUCTION

Viburnum opulus belongs to genus of *Viburnum* L, member of Adoxaceae family (formerly known as Caprifoliaceae) and could be included in the monotypic family of Viburnaceae. Commonly, the plant is known as guelder rose in Europe and as Gilaburu in Turkey (Karaçelik et al., 2015; Özrenk et al., 2011; Sagdic et al., 2014; Velioglu et al. 2006, Akbulut et al 2008; Kajszczak et al. 2020). The natural habitats of the plant are Europe, Northwest Africa, Turkistan (Davis, 1972) and Canada (Richard & Pierre, 1992).

In recent years, the beneficial effects of phenolic compounds on human health have led to an increased interest in edible naturally occurring sources rich in these compounds (Hooper & Cassidy, 2006). These polyphenol constituents in the plant tissue are affected by numerous exogenous factors such as environmental parameters including ultraviolet (UV) radiation, time of harvest, and damage caused by pests as well as competition with other individuals/species, in addition to genetic or age-related factors. These compounds are also found to be well correlated with antioxidant potential, which generally increases with an increase in the number of hydroxyl groups that they bear and decrease in their glycosylation (Katalinic et al., 2004). The presence of these phenolic compounds give rise to a wide range of medicinal properties such as antiallergic, anti-artherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodialatory

effects. It was also known that natural sources of these phenolic compounds exhibited stronger antioxidant activity than synthetic ones. The fruits of *V. opulus* have medicinal properties due to its vitamins, minerals, antioxidants and other bioactive substances (Rop et al. 2010; Kim et al. 2003; Andreeva et al. 2004; Cam et al. 2007; Velioğlu et al. 2006; Altun et al. 2008). *V. opulus* is used in folk medicine to treat colds, cough, ulcers, diabetes, tuberculosis, hypertension and liver diseases (Altun et al. 2009; Soylak et al. 2002; Al et al. 2017; Eryılmaz et al. 2013).

Different plant parts of *V. opulus* displays different constituents; for instance, dried fruits (Sagdic et al. 2006), fresh fruit (Turker & Yildirim, 2015) and seed oil (Yilmaz et al. 2008) have been reported to display antimicrobial activity. Moreover, fruits display anti-inflammatory (Zakłos-Szyd et al., 2020) antidiabetic (Zakłos-Szyd et al., 2015), anti-obesity (Podsedek et al., 2020) and anti-cancer properties (Ucar et al., 2012; Kajszczak et al. 2020). Moreover, phenolic acids, proanthocyanins and anthocyanins (Van et al., 2009; Zayachkivska et al., 2006; Deineka et al., 2005; Turek & Cisowski, 2007) flavonoids and total phenolics (Rop et al., 2010; Velioglu et al., 2006) were determined in fruits of *Viburnum* species which ensure medical effects and usage in food preserving (Česonienė et al., 2012).

Traditionally various anatomical parts of *V. opulus*, including bark, leaves, flowers and fruits have been used for food and medicinal purposes in Europe and Asia (Kraujelité et al, 2013). So far, most of the research have been carried out to characterize the chemical composition

of *V. opulus* fruit. However, there a few data or little is known about the chemical characteristic of other parts of the plant for which health-promoting effects have also been demonstrated (Polka et al., 2019).

In this study, total phenolic content and antioxidant activity of guelder rose (*Viburnum opulus* L.) were investigated regarding different plant parts and altitudes.

1. MATERIAL AND METHOD

1.1. Plant Material

The plant material of our study consisted of different parts (bark, fruit, leaf) of the *Viburnum opulus* L. plant collected from different altitudes (1210 m, 1220 m, 1280 m, 1380 m) from flora of Trabzon.

Table 1: Altitudes and coordinates of *V. opulus* collected from flora of Trabzon

Plant species	Altitudes (m)	Date of collection	Coordinates
Viburnum opulus	1220	19/09/2019	40°49'43"N 39°19'20"K
	1210	19/09/2019	40°49'43"N 39°19'22"K
	1280	19/09/2019	40°48'17"N 39°19'11"K
	1380	19/09/2019	40°49'43"N 39°19'10"K

Figure 1. Guelder rose (*Viburnum opulus* L.) in its natural habitat in Trabzon, Turkey.

1.2. Pretreatment for Analysis

Dried and fresh fruits of guelder rose were extracted during 8 hours using water and 70% methanol as solvent. The resulting extracts were condensed in the rotavapord (40-45º C) under vacuum. All extracts were stored at +4ºC until the moment of analysis.

Figure 2. Fresh and dry samples of guelder rose obtained from fruit, bark and leaf parts

1.3. Determination of Total Phenolic Content

Total phenols in the extracts were calculated as equivalent to gallic acid (GAE) using Folin-Ciocalteu method. 50 µL of sample solution and 250 µL of Folin-Ciocalteu reagent were added into a 10 mL graduated container containing 3.95 mL of distilled water. After 1 minute, 750 µL of 20% aqueous Na_2CO_3 was added and it was completed with 10 mL of water. As a control, reagent mixture without extract was used. After 2 hours of incubation at 25° C, the absorbance was measured at 760 nm and compared with the gallic acid calibration curve. The total amount of phenolic substance was calculated as equivalent to gallic acid. Three parallel experiments were made and the results were given as average values.

1.4. Determination of Antioxidant Activity

Antioxidant activity A modified version of the FRAP assay described by Izzreen & Fadezelly (2013) was used to determine the antioxidant activity of collected samples as mg $FeSO_4$/gr DW. For the determination of antioxidant content of the samples as pretreatment, 0.1 g of each dried sample was completed with methanol (80 %) to reach 10 ml volume. Samples were mixed first in the water bath (50°C) for a duration of 20 minutes and the samples were keep waiting after this procedure for 1h in the dark. The mixture was centrifuged after that for a 20 min, 4000 cycle/min process for obtaining the extracts, which are used for the determination of phenolic content and antioxidant activity of the investigated samples. Collected samples were analyzed regarding

their antioxidant activity values. Green tea leaves were collected at two shooting periods and the leaves were dried in the drying oven at 40°C and its antioxidant activity was determined using the UV-spectrophotometer by the FRAP method. The determination of antioxidant capacity of investigated samples (pretreatments completed) was done using the FRAP method. The FRAP method bases on the colorization after the degradation of the Fe^{+3} ion, bounded to TPTZ in an acid environment, to Fe^{+2}. 300 mM acetate buffer (pH 3,6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ solutions were mixed at a proportion of 10:1:1 as FRAP (ferric reducing / antioxidant power) reactive to obtain a buffer solution. A $FeSO_4.H_2O$ solution was used to prepare different standard probes to obtain a calibration curve. The final samples were obtained with a mix of 1980 µl FRAP dispersive + 20 µl sample and keep waiting after that for 3 min in an ultrasonic shaker (50°C). The measurements were done using a UV Spectrophotometer device at a wave length of 595 nm to obtain the final absorbance values. The reagent mixture without extracts and BHT were used as controls. After incubating at room temperature and in the dark for 30 minutes, absorbances were read at 517 nm and the percentage of inhibition was calculated using the following equation;

$$\% \text{ inhibition} = [(\text{Abscontrol} - \text{Abssample}) / \text{Abscontrol}] \times 100$$

Values are given as the average of three parallel experiments.

1.5. Data Analysis

Correlation analysis was performed to clarify the relationship between investigated data and principal component analysis (PCA) was carried out to elucidate their relationships by using the statistical software package XLSTAT2010 Trial Version. PCA analysis is the two-dimensional visualization of the position of investigated accessions relative to each other. The principal components represent the axes which are the orthogonal projections for the values representing the highest possible variances in the case of PC1 and PC2. The obtained data were used to create scatter plot diagrams (Backhaus et al. 1989). Therefore, a factor analysis was performed, whereby each variable was used to calculate relationships between variable and investigated factors. Based on the obtained data the cluster dendrogram was created.

2. RESULT AND DISCUSSION

Total Phenol content, antioxidant values and of bark, leaf and fruit samples of *Viburnum opulus* collected from different altitudes in Trabzon their standard deviations are given in Table 2. These determined characters will be discussed in detail.

2.1. Total Phenolic Content

The total phenol content of the plant was as follows; it ranged from 86.395 mg GAE / g to 124.792 mg GAE / gr regarding all altitudes and plant parts. In terms of different plant parts, the minimum- maximum Total Phenol Content values of bark, leaf and fruit parts were

determined respectively as 107.451 - 116.122 mg GAE / gr, 86.395 - 124.173 mg GAE / gr and 117.360 - 124.792 mg GAE / gr. The highest total phenol content was obtained in the leaf part (124.792 mg GAE / gr) at 1220 m altitude and the lowest in the fruit part (86.395 mg GAE / g) at 1280 m altitude (Table 2, Figure 3).

Table 2: Total Phenolic content and antioxidant values of bark, leaf and fruit samples of *Viburnum opulus* collected from different altitudes in Trabzon

V. opulus samples collected at different Altitudes	Total Phenolic Content	Standard deviation	Antioxidant activity	Standard deviation
1210 m Bark	110.548	± 0.122	93.54	± 0.015
1211 m Fruit	109.9	± 0.197	93.136	± 0.231
1212 m Leaf	118.0	± 0.058	90.31	± 0.603
1220 m Bark	107.5	± 0.623	94.482	± 0.608
1220 Fruit	124.2	± 0.693	92.194	± 0.088
1220 Leaf	124.8	± 0.327	81.689	± 0.062
1280 m Bark	116.122	± 0.307	92.463	± 0.188
1280 Fruit	86.395	± 0.439	91.79	± 0.504
1280 Leaf	119.218	± 0.093	92.463	± 0.253
1380 m Bark	111.787	± 0.251	91.925	± 0.661
1380 Fruit	90.111	± 0.088	94.213	± 0.248
1380 Leaf	117.36	± 0.377	92.059	± 0.106

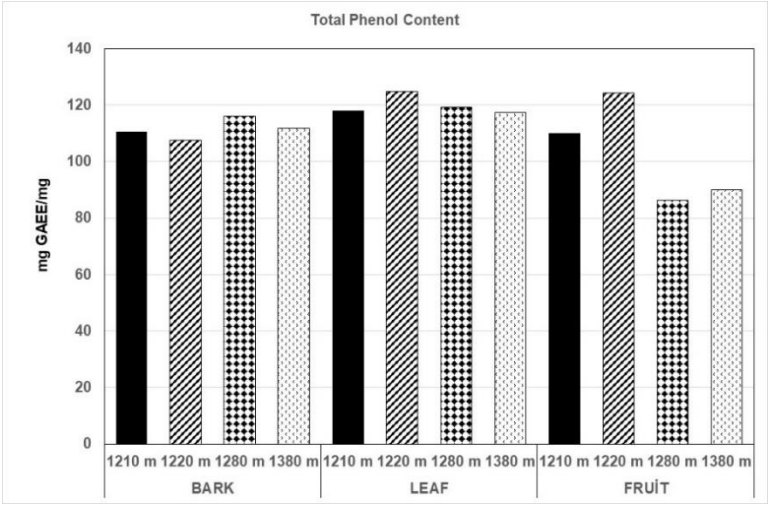

Figure 3. Total Phenol Content of bark, fruit and leaf plant parts at different altitudes

2.2. Antioxidant Activity

Using DPPH radical scavenging method, the antioxidant activity content varied between 81.69-94.482 %. Inhibition values (%) indicating the amount of antioxidant activity was determined in the bark part as 91.925-94.482 %, in the fruit part as 91.790-94.213 %, in the leaf part as 81.696-92.463 %. The highest antioxidant activity was determined in the bark part (94.482%) at an altitude of 1220 m, and the lowest antioxidant activity in the leaf part (81.696%) at an altitude of 1220 m (Table 2, Figure 3).

The plant's antioxidant compounds are mainly phenolic and include compounds such as tocopherols, carotenoids, phenolic acids (benzoic acid derivatives and cinnamon acids), flavonoids, and dipropenes (Shahidi, 1997). Secondary plant-derived metabolites, including

phenolic compounds, have a potent potential to clear free radicals that exist in all parts of the plant, such as the leaves, fruits, seeds, roots, and skin (Mathew & Abraham, 2006).

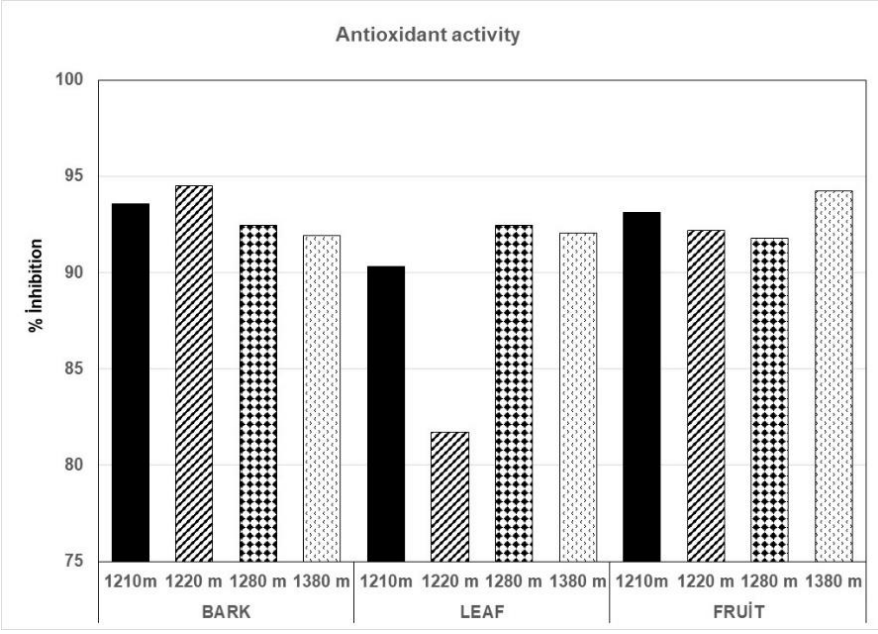

Figure 4. Antioxidant activity of bark, fruit and leaf plant parts in different altitudes

2.3. Principal Component Analysis

Principal component and cluster analyses are favored means for characterization of genotypes and their grouping on similarity (Peeters & Martinelli 1989; Mohammadi & Prasanna 2003). PCA is a beneficial statistical tool for differentiation of plant materials giving information on the variation in chemical content/composition of several species (Smelcerovic et al. 2008, Bertoli et al. 2011, Cirak et al. 2016 a,b). Combination of the two statistical tools provides broad information of

the traits making significant contributions to genetic diversity in crops (Malik et al. 2014). Biplot is another widely utilized procedure for graphical displaying of accession groups with the aim of searching the relationships among agro-morphological characters in several cultivars (Aghaee et al. 2010). In the present study, we used the above-mentioned statistical tools to evaluate difference of bark, leaf and fruit parts *V. obulus* collected from different altitudes.

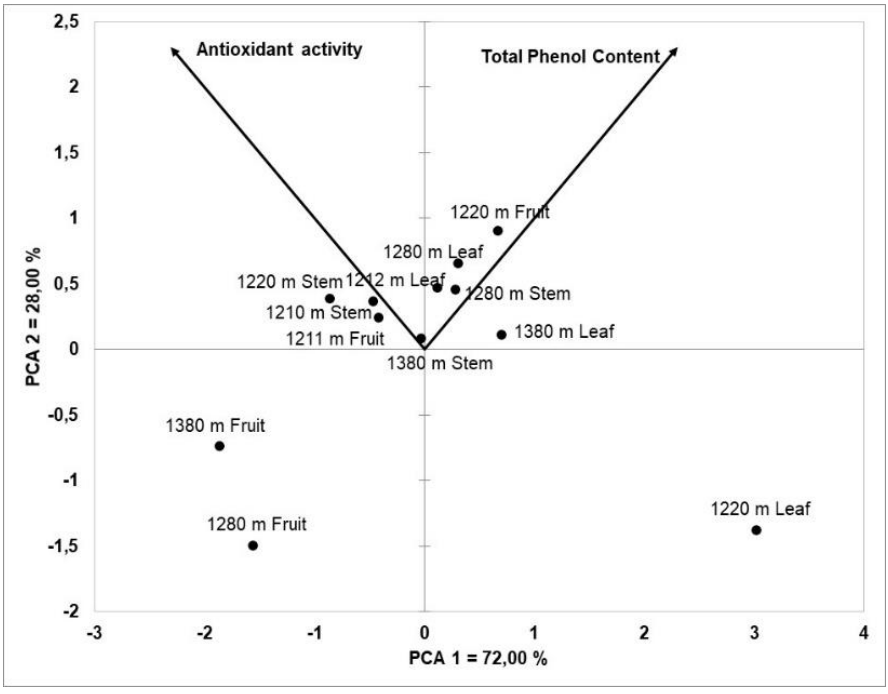

Figure 5: Principal Component Analysis of *V. opulus* bark, fruit and leaf plant parts collected from different altitudes in Trabzon

Principal Component Analysis revealed that bark, leaf and fruit parts of *V. opulus* collected from different altitudes could be differentiated based on their total phenol content and antioxidant capacity. The first two principal components corresponded to 100 % of the total variation (PC 1 = 72 %, PC 2 = 28 %) regarding determined characters in the investigated material. Specially, the leaf parts of *V. opulus* collected from altitude 1220 in Trabzon demonstrated a different total phenolic content and antioxidant capacity compared with rest samples (Figure 5 and 6). Further, fruit samples collected from the altitudes 1280 and 1380 m displayed different total phenolic content and antioxidant capacity. Leaf samples from 1212, 1280 and 1380 m, bark samples from 1280 m and fruits from 1220 displayed a different total phenol content.

The altitude of plant growing environment is an important environmental factor influencing the composition and quantity of bioactive compounds in plants (Khalil et. al, 2020). Despite the numerous studies on the altitude effects on plant content of bioactive constituents (Khalil et al., 2020; Rieger et al., 2008; Spitaler et al., 2008; Gulzar et al., 2017) there is no published research and information on the altitudinal relationship of total phenols and antioxidant capacity of *V. opulus*. Our results revealed that the total phenol content and antioxidant capacity of bark, leaf and fruit parts of *V. opulus* were effected by different altitudes collected from flora of Trabzon.

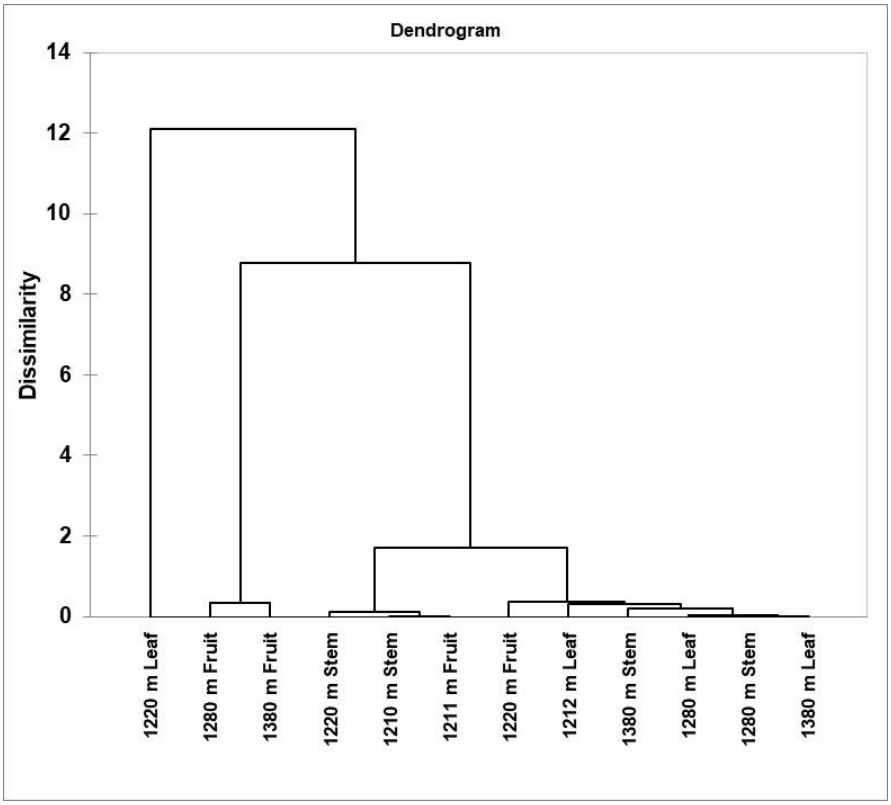

Figure 6: Dendogramme of bark, fruit and leaf plant parts in *Viburnum opulus* at different altitudes

3. CONCLUSION

The total phenolic content and antioxidant activity of guelder rose (*V. opulus* L.) regarding its bark, leaf and fruits collected from different altitudes were investigated. The amount of total phenolic content and antioxidant activity changed due tor altitude and plant parts. Guelder rose (*Viburnum opulus* L.) fruit has many health benefits as explained in beginning of the presentation. Although, the plant is not recognized

well by the people of the region and is not used commercially. As a result of our study, guelder rose, which has an important potential in the region, was investigated and its antioxidant and total phenolic content was revealed.

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CHAPTER 13

IN VITRO ANTIOXIDANT AND NUTRITIONAL CONTENT VALUES OF GOJI BERRY (*Lycium barbarum* L.)

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INTRODUCTION

Goji berry (*Lycium barbarum* L.) is a perennial plant belonging to the Solanaceae family (Potterat, 2010). Originating from the Asian continent, this plant is now grown in different parts of countries such as Japan, Korea, Taiwan, and China (Sharamon, 2007; Kulczyński, 2016). Goji berry fruits are orange-red in color, rich in vitamins such as beta carotene, C, B-complex and E, and about 19 amino acids, carotenoids, zeaxanthin, lutein, such as Ca, K, Fe, Zn mineral substances (Kulczyński, 2016; Yılmaz & Kınay, 2016). Fruits can be consumed directly as well as in making fruit juice and marmalade (Yılmaz, 2013). Reid (1995) and Zhufan (2000) stated that goji berry is therapeutic in liver, kidney, and lung diseases. Goji berry is also a powerful antioxidant and has effects on cardiovascular and cancer diseases besides its anti-aging effect (Guo et al., 2008; Kabakcı, 2013; Kulczyński, 2016).

Oxygen-centered free radicals can oxidize lipids, proteins, and DNA, causing tissue damage and subsequent cell death (Boran & Uğur, 2017; Ozsoy et al., 2008; Caro et al., 2019). Most of the phenolic compounds found in medicinal and aromatic plants have a protective effect against oxidative stress caused by free radicals. These components have preventive effects against cancer and cardiovascular diseases (Oreopoulou et al., 2019; Albayrak et al., 2010; Soory, 2009; Amin & Bano, 2018; Liguori et al., 2018; Liu et al., 2018).

Nowadays, alternative medicine gains importance when modern medicine is insufficient or when drugs cannot be used due to side

effects, and as a result, an increase in the use of medicinal and aromatic herbs is observed. Goji berry has become a plant that is sought after and preferred by people thanks to its properties. In this context nutritional contents and antioxidant activity of goji beri were investigated.

1. MATERIAL AND METHOD

1.1. Obtaining Extracts and Chemical Composition

Goji berry plants were grown in Sivas Cumhuriyet University, Sivas Vocational School, Plant and Animal Production Department, on trial plots. Samples were taken from Goji berry plants and dried in the oven. The plants were then powdered with a laboratory grinder. The powdered plant materials were macerated with ethanol. After one day of agitation in the shaker, the plant particles were filtered, and dried in an oven to obtain the extracts. The extracts were analyzed by Gas Chromatography / Mass Spectrometry (GC-MS) for determine their components and relative percentages (Sacchetti et al., 2005).

1.2. Biological Activity Evaluation

1.2.1. *In vitro* antioxidant activity

The DPPH radical scavenging activity of the extracts was evaluated according to the Blois method (1958) with slight modification. ABTS radical scavenging activity was evaluated by the method of Re et al. (1999) with minor modifications. Total phenolic content was determined with spectrophotometric method (Clarke et al. 1993) and expressed as gallic acid equivalents and flavonoid content was determined with the aluminum chloride colorimetric method of Molan

& Mahdy (2014). The content of total flavonoids was expressed as milligrams of catechin equivalent per gram of the dry weight of the extract.

1.3. Macro and Micro-Nutrient Contents

First of all, the samples were grinded and made ready for analysis. Later, the determination of N content was performed by the modified Kjeldahl method (Bremner, 1965). In order to determine the contents of P, K, Fe, Mn, Zn and Cu, 5 ml of 65% nitric acid and 2 ml of 35% hydrogen peroxide were added to the container of the sample burning unit. After the samples were disintegrated, they were filtered through filter paper with a blue band and then the solution volume was made up to 20 ml with ultrapure water. The amounts of P, K, Ca, Mg, Mn, Fe, Cu, and Zn were determined using atomic absorption spectrometry (Gesto-Seco et al., 2009, Bremner, 1965, Murphy & Riley, 1962).

2. RESULTS AND DISCUSSION

2.1. The Chemical Composition

GC-MS was used to identify the components of the extracts and Gas Chromatography was used to determine the relative percentages (Sacchetti et al., 2005). The chemical composition of the ethanol extracts of Goji berry was evaluated. According to the obtained data, total of 21 components were determined. The major component was exhibited “Tributyl acetylcitrate” (17.44 %), followed by “Hexatriacontane” (7.96 %), beta.-D-Glucopyranoside, methyl (CAS) (7.28%) and Decanedioic acid, dibutyl ester (6.76%) (Table 1).

Table 1: Chemical components of ethanol extracts of Goji berry

Peak no	Retention time	% Area	Compound name
1	7,836	3,36	Maltol
2	9,834	4,22	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
3	12,269	2,15	1,2-Ethanediol, 1-(2-furanyl)- (CAS)
4	12,441	3,37	5-Hydroxymethylfurfural
5	19,345	1,27	3-Mercaptohexyl acetate
6	19,477	2,99	5-(1,2-Dihydroxyethyl)dihydrofuran-2-one
7	23,025	2,18	1,4-Anhydro-d-mannitol
8	23,392	2,79	Decanoic acid (CAS)
9	23,699	7,28	beta.-D-Glucopyranoside, methyl (CAS)
10	23,955	1,91	Piperidine, 1-(1-cyclopenten-1-yl)- (CAS)
11	24,165	3,83	3-Deoxy-d-mannoic lactone
12	24,515	1,79	Phosphonic acid, (1-methylethyl)-bis(2-ethylhexyl) ester
13	24,664	1,73	Isosorbide Dinitrate
14	33,213	1,51	alpha.-D-Mannofuranoside, 1-O-decyl-
15	38,635	2,08	1-Propene-1,2,3-tricarboxylic acid, tributyl ester
16	38,758	6,76	Decanedioic acid, dibutyl ester
17	39,241	0,75	Butyl citrate
18	39,385	4,59	Tetracosane
19	40,853	17,44	Tributyl acetylcitrate
20	45,193	7,96	Hexatriacontane
21	52,847	1,85	Tetrapentacontane

2.2. Biological Activity Evaluation

2.2.1. *In vitro* Antioxidant Activity

2.2.1.1. DPPH Radical Scavenging Activity (%)

The antioxidant activity of Goji berry (*Lycium barbarum* L.) was tested by DPPH and ABTS radical scavenging method (Figure 1).

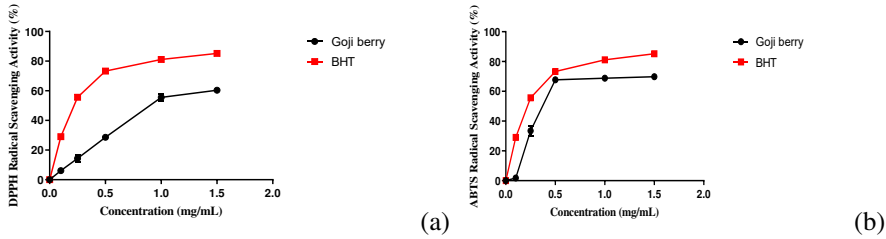

Figure 1. DPPH (a) and ABTS (b) radical scavenging activity of ethanol extract of Goji berry (*Lycium barbarum* L.)

The free radicals that resulting from the functions of cells, cause vital disease such as cancer, diabetes mellitus, and hypertension. Antioxidants are effective for the elimination of free radicals. Therefore, natural antioxidants are important. In this study, goji berry fruits are extracted with methanol and their antioxidant activity has been evaluated by radical scavenging assays. According to obtained data, goji berry fruits have high levels of antioxidant activities at the base of DPPH and ABTS radical scavenging activity (the IC_{50} values; 1.09±1.5 µg/mL and 0.76±1.28 µg/mL, respectively). These data have been observed were very close to standard BHT (the IC_{50} value is 0.479±0.6 µg/mL) (Figure 1). According to Mocan et al. (2015) *Lycium barbarum* has moderate antioxidant activity. Yan et al. (2014) investigated that the antioxidant activity of different organs of goji

berry. According to their report, the fruits of goji berry showed the strong antioxidant activity than other organs.

2.2.2. TFC (Total Flavonoid Content) and TPC (Total Phenol Content)

The total phenol and total flavonoid content of ethanol extract from Goji berry (*Lycium barbarum L.*) are presented in Figure 2.

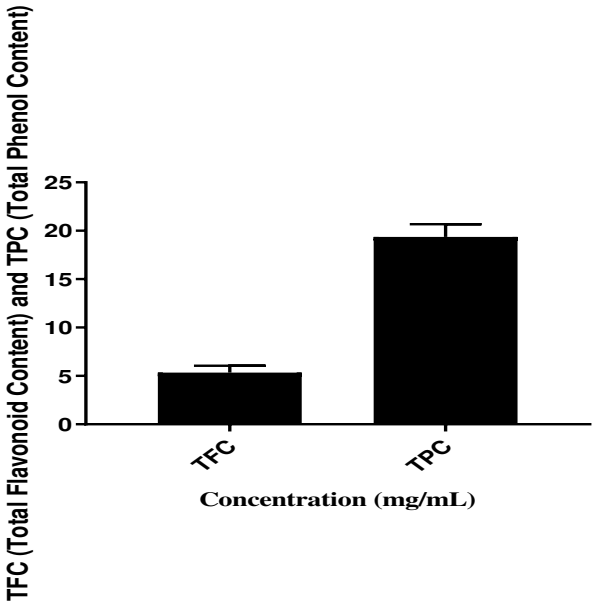

Figure 2. TPC and TFC of ethanol extracts of Goji berry (*Lycium barbarum* L.)

The total phenol content (TPC) was higher found than total flavonoid content (TFC) (19.36 ± 1.3 mg GAE/g and 5.3 ± 0.7 mg CE/g, respectively) (Figure 2). Plants can inhibit free radicals due to the high amount of total phenolic and flavonoid compounds they contain. According to Yan et al. (2014) report that goji berry fruits contain phenolic compounds.

2.3. The Macro and Micro-Nutrient Contents

Table 2: The Macro and Micro-nutrient Contents of Goji berry

Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	K (%)	Ca (%)	Mg (%)	P (%)	N (%)
27.2±0.8	243.9±8	42.5±6.6	15.4±2.3	2±0.2	0.2±0.04	0.4±0.03	0.8±0.1	6.1±0.3

As a result of the analysis on the nutritional content of the goji berry plant, this plant contains macro elements such as 6.1% N, 0.8% P, 2% K, 0.2% Ca and 0.4 % Mg and it has the micro elements such as 27.2% Mn, 243.9% Fe, 42.5% Zn, 15.4 % Cu (Table 2). According to the study of another researchers, Goji berry has high level of P, K, Ca, Mg, Fe, Mn, Se, Zn, and Al (Yan et al. 2014).

3. CONCLUSION

Goji berry has been observed that have a high antioxidant potential. In the same time, results showed that it can be a good food sources for humans thanks to its rich nutritional contents. In this context, it can be said that the consumption of this plant can be beneficial for health.

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CHAPTER 14

EFFECTS OF *Papaver somniferum* L. ON CANCER

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INTRODUCTION

Papaver somniferum L., also known as poppy, is a versatile plant that produces a large part of benzylisoquinoline alkaloids such as morphine, codeine, noscapine, which are narcotic and analgesic, and which is also used for medicinal purposes or as an ornamental plant (Gültepe, 2013; Ghafoor et al., 2019).

General Properties of *Papaver somniferum* L.

Opium Poppy (*Papaver somniferum* L.) is one, two or perennial herbaceous plant from the poppies family (Papaveraceae). The leaves are differently segmented and tapered towards the tip, while the base of the leaf surrounds the stem. Its edges are bluish, green or grayish green.

The flowers are on the long stalk and at the ends of the branches, usually 4 in number, and may be blue-purple, white, pink-red. As soon as the flower opens, two calyculus are shed. It contains four petals and fertilization dusts before it blooms. After the flowers wither and fall, the middle core grows and takes the form of a capsule and a sphere. The capsule is 4-5 cm in diameter. Seeds are in capsules. It is 50-150 cm long.

Thanks to the polyunsaturated fatty acids such as linoleic, oleic and palmitic acid, minerals and various phenolic compounds, poppy seed oil has many beneficial effects on health such as lowering the level of cholesterol in the blood and preventing cardiovascular diseases (Singh & Sharma, 2020).

Since the analgesic substances obtained from the plant are of narcotic importance, they are allowed to be produced in certain regions and in a controlled manner. Turkey and India traditional poppy production while in Australia, France, Spain and Hungary supervised by the United Nations as a commercial poppy production is done (TMO, 2021).

Poppy plant has economic and commercial value in terms of both seed and capsule. It is known that the poppy capsule contains about 30 different alkaloids as well as the main alkaloids of medical importance such as morphine, codeine, thebaine, noscapine and papaverine (Facchini et al., 1995; Gürkök et al., 2010; Da Cheng et al., 2015; Dilek et al., 2018) (Table 1).

Table1: Medicinally important alkaloids in poppy plant

Alkaloid	Average occurrence rates in poppy	Medicinal Importance
Morphine	%5-25	Narcotic, Analgesic
Noscapine	% 2-10	Antitussive , anticancer
Codein	% 0,5-3	Narcotic, Analgesic, Antitussive
Thebaine	% 0.2-1	Sedative, Antitussive
Papaverine	%0,5-1,3	Antispasmodic, Vasodilator

Anticancer Property of Alkaloids

Cancer is a global disease that can be seen in all tissues and affects the whole organism with its metastatic feature and has a very high mortality rate. Although it is known that there are many genetic, environmental and chemical reasons that affect the formation of the

disease, it seems very difficult to develop a treatment. Chemotherapy, radiotherapy and surgical intervention are applied in the treatment of this complex disease.

Today, it is known that various drugs are used in the chemotherapeutic treatment process of this disease, which are synthetic-containing or obtained as a result of the hybrid of natural compound and synthetic molecules (Sivaraj et al., 2014). However, studies on the use of natural agents in order to reduce side effects and provide a more efficient treatment for the patient have gained momentum, especially in developing countries. For this purpose, alkaloids, polyphenols and taxols obtained from plants are being investigated for therapeutic use.

When the anticancer activity of polyphenols was examined, it was determined that they had apoptosis-inducing properties just like noscapine. The key role in this mechanism appears to be the mobilization of Cu ions that bind to chromatin, which induces DNA fragmentation (Azmi et al.,2006).

A large part of polyphenolic compounds are composed of flavonoids and there are many studies showing that these secondary metabolites have anticancer effects in vitro. Some of the cancer cells it affects; human lung cancer (A456), hepatoma (Hep-G2), cervical carcinoma (Hela) and breast cancer (MCF-7) are human leukemia (HL-60) (Cao et al., 2013; Kumar et al., 2014; Wen et al., 2014).

Humans have used alkaloids for many years as medicines, ointment and poisons. The physiological effects of some alkaloids are clearly

known. For example, quinine is used in the treatment of malaria, morphine in the relief of severe pain. Most alkaloids can be toxic to humans when overdosed.

It is known that some of the alkaloids (evodiamine, piperine, amptothecin, sanguinarine, vincristine, vinblastine, berberine, noscapine), which are densely found in generally Papaveraceae, Loganiaceae, Leguminosae and Menispermaceae families, have a very strong effect as chemotherapeutic agents (Huang et al., 2011). Apart from the pure forms of these alkaloids, it can be said that their analogues also have strong cytotoxicity and apoptotic effect. For example, it has been observed that when 9-bromo-noscapine, which is a noscapine analogue, is applied with nano-structured lipid particles in lung cancer cells, it has a higher cytotoxic effect and induces apoptosis compared to the free drug used in routine therapy (Jyoti et al., 2015; Mondal et al., 2019).

Along with other herbal therapeutic agents, the anticancer activities of alkaloids have a very high potential for drug development. Studies on this subject show that among these alkaloids, noscapine derived from *Papaver somniferum* L. is an important anticancer agent.

Anticancer Effects of *P.somniferum* L.

Studies investigating the antiproliferative and anticancer effects of *P.somniferum* L. extract in vitro conditions are limited. In one of these studies, the anticancer activity of the poppy on hexane, ethyl acetate, methanol extracts and HT29, HeLa, C6 tumoral cells and non-tumoral

Vero cell line were examined and it was found that the most cytotoxic activity was in the ethyl acetate extract of the poppy stalk and the lowest cytotoxic effect was in the poppy capsule. While the part of the poppy plant with the highest alkaloid density is capsule, this result obtained from the study reveals that the use of direct extract without making alkaloid fraction from the plant is weak in showing anticancer activity (Güler et al., 2016).

In another study investigating the anticancer effect of *P. somniferum*'s methanol extract on 5 different cancer cell lines (CEM / ADR 5000, MCF-7, Caco-2, CCRF-CEM), CEM / ADR 5000 and CCRF, which are especially multidrug resistant. It has been reported that CEM cell lines correlate strongly with each other and all cell lines undergo a strong inhibition by the alkaloid extract of *P.somniferum* (Sharopov et al., 2018).

Anticancer Effect of Noscapine Alkaloid

It has been stated in many studies that noscapine (Figure 1), one of the alkaloids found in *Papaver somniferum* L. plant, has anticancer properties as well as cough suppressant properties. In these studies, it is seen that the alkaloid itself, its analogs or its combined forms with a different substance were used (Table 2).

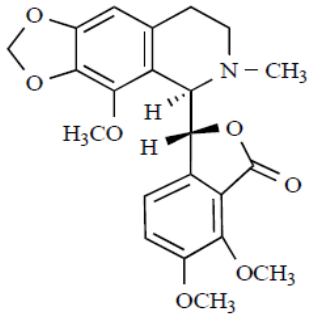

Figure 1. Chemical structure of Noscapine (Mahmoudian and Rahimi-Moghaddam, 2009).

Table 2. In vitro anticancer effects of noscapin on different cancer cell lines.

Cell Line of Affected	**Effect Mechanism**	**Reference**
MCF-7, MDA-MB-231	NF-κB activation inhibitor, apoptosis inducing	Quisbert-Valenzuela et al., 2016
HeLa, E.G7-OVA, MCF-7	Tubulin subunits binding, mitosis arresting in tumoral cell	Ye et al., 1998
LoVo/5-FU, HT29/5-FU	Regulation of Warburg effect via PTEN and mitochondria damage, apoptoz inducing	Tian et al, 2020.
A549 and H460	It enables decrease of pAkt, Akt, cyclin D1, survivin, PARP, Bcl2 expression and activation of multiple signaling pathways including apoptosis with cisplatin..	Chougule et al., 2011
CEM, CEM/VLB100, CEM/VM-1-5, 1A9, 1A9/PTX22	A nitro-analog of noscapine, 9-nitro-noscapine, progression of cell cycle by mitotic arrest	Aneja *et al., 2006a*
MCF-7, MDA-MB-231, BT-474, SK-Br3, T47D, and ERMDA-MB-231	Noscapine analog EM015, regresses breast tumor xenografts	Aneja *et al., 2006b*
Murine B16LS9	Arrested in mitosis	Landen et al.,

		2002
1A9, 1A9PTX10, 1A9PTX22	c-Jun NH2 terminal kinase (JNK) induces appotosis by activation	Zhou et al., 2002
U-87	haloderivatives of noscapine 9-halonoscapines 2 is cytotoxic then noscapine	Verma et al., 2006
HCT116 cells: p53+/+ (p53-wt), p53-/- (p53-null), p21-/- (p21-null), and BAX-/- (BAX-null).	Apoptosis is induced with increasing p53expression	Aneja et al., 2007
H460 NSCLC	Decreasing in xenografted tumor volüme by up regulation of PARP, Bax, caspase-3 and repression of Bcl2 expression.	Jackson et al., 2008
LNCaP and PC3	Inhibition of cell growth with paclitaxel and noscapine combination	Rabzia et al., 2017
LN229, A172 U251	TMZ-resistant glioma cells are inhibited growth with treating noscapin	Jhaveri et al., 2011
HeLa, MIA PaCa-2, SK-N-SH, and DU145	It has the potential to inhibit tubulin protein in MIA PaCa-2 cells with analog of nos.	Nagireddy et al., 2019

Biological activities of *P.somniferum* L. on Cancer

Other factors that indirectly affect the activity of *Papaver somniferum* L. on cancer are its antimicrobial, antioxidant, analgesic and apoptotic properties.

When the antimicrobial properties of poppy are examined, it was determined that the water extract obtained from the seeds of the poppy plant grown in Pakistan has an antimicrobial effect on *Alcaligenes spp., Citrobacter spp., E. coli, Micrococcus roseus*. (Chaudhry &

Tariq, 2008). In another study, it was specified that poppy flower essential oil showed antimicrobial activity on *M. luteus*, *Proteus vulgaris* and *Klebsiella pneumonia* (Dilek et al., 2018). The blue poppy seeds grown in Turkey in a study of the antimicrobial activity of the oil, the most sensitive microorganisms against the blue seed oil was determined that *E. coli* and *L. monocytogenes* (Yücel Şengün et al., 2020). It is seen that extracts obtained from different parts of the plant have different antimicrobial effects. With the increase of studies on this subject, it can reveal whether the antimicrobial activity increases the anticancer effect of poppy.

Free radicals are immune system suppressing agents that increase the progression of cancer. It can be said that antioxidants are quite effective in inhibiting these molecules. When the antioxidant activity of the poppy plant was examined, it was revealed that the alkaloid extract had a very strong antioxidant effect. In addition, the cytotoxic effect of alkaloid extract was also investigated in the same study and it was observed that it provided low expression of ABC (ATP-binding cassette) transporter (Sharopov et al., 2018). ABC proteins are expressed in many tumor tissues as well as in healthy tissues. These carriers exclude antineoplastic drugs from tumor cells, preventing the drug from accumulating in the tumor tissue, thus leading to the failure of the administered chemotherapy.

Due to its morphine alkaloid, one of the main activities of the poppy plant is its analgesic feature. Morphine is considered the "gold standard" for pain relief and is currently one of the most effective

drugs clinically available for alleviating severe pain associated with cancer. It has also been suggested that it may be a regulator of tumor growth (Bimonte et al., 2015)

Agents with the potential to induce apoptosis can be considered good candidates for cancer therapy due to their effects on the uncontrolled proliferation of malignant cells. Although there is generally evidence that noscapine exhibits anticancer activity, studies have shown that papaverine alkaloid also induces apoptosis (Gao et al., 2002; Afzali et al., 2015). In addition, the cytotoxic effect of Papaverine and some of its analogues has been detected in breast cancer, melanoma and prostate cancer (Rubis et al., 2009).

Further studies on non-toxic alkaloids such as noscapine and papaverine may enable the use of these alkaloids as chemotherapeutic agents in cancer treatment.

It is obvious that the poppy plant is an important therapeutic agent that should be used in cancer treatment, especially considering the anticancer effect of the noscapine alkaloid. In addition, when the antimicrobial, antitussive, antioxidant and analgesic properties of the benzylisoquinoline alkaloids contained in the plant are evaluated, it shows how rich it is in medical terms.

It is predicted that the metabolites to be obtained from the *Papaver somniferum* L. plant, which has a rich content in terms of both commercial, economic and health, have a promising potential for the treatment of many diseases, especially cancer, as a result of future.

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CHAPTER 15

COLORING CHARACTERISTICS AND FASTNESS DEGREES OF LICORICE (*Glycyrrhiza glabra*)

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INTRODUCTION

The use of natural dyes in textiles from the beginning of human history to the beginning of the 19th century was first introduced in 1856 by W.H. With the discovery of "Mauveine", the first synthetic dyestuff by Perkin, it has gradually been replaced by synthetic dyestuffs. (Tarakçıoğlu, 1983).

Until the invention of synthetic dyestuffs, natural dyes were used in the fields of textiles, food, medicine and cosmetics that directly affect the human body. Synthetic dyes have quickly replaced natural dyes due to reasons such as low cost, offering a very wide color scale, making the applied procedures more effortless and in a short time. Synthetic dyestuffs are used not only in clothes, but as food dyes everywhere today. Since it has been used for more than a century, its negative effects on human health have started to emerge and the return to natural dye has started, especially in developed countries.

Like many other industries, textile dyehouses strive to improve their sales performance by offering an alternative product range for their customers. Particularly, environmentally conscious consumers accelerate this process by examining the production processes of the textile materials they use and taking care to choose environmentally friendly products. Manufacturers enrich at least some, if not all, product pallets with products that we can describe as special production (Benli, 2020).

Although it is troublesome in entire world due to its other superior features, there is a return to nature in every subject. As in other fields, the trend of returning to nature in textiles has increased the importance of natural dyes in textile, especially in carpet and rug dyeing (Özbek, 1996).

In the study, dyeing studies using different mordants with the licorice plant grown in other regions of our country, especially in the south-eastern Anatolia region, are presented. In addition, the degrees of friction, light and water drop fastness, which are very necessary for textile products, were measured.

1. PROPERTIES OF LICORICE (*Glycyrrhize glabra*) FROM MEDICAL AROMATIC PLANTS

There are around ten thousand plant species in our country and about three thousands of them are endemic. It is accepted that up to 1000 of these plants are used for medicinal purposes (Arslan et al., 2000).

5 species of licorice root, which is a member of the Fabaceae (Legumes) family, grow in our country, but one of them has medicinal value. The species that spread in our country; *Glycyrrhiza glabra* L. var. *glandulifera* (Waldst et Kit.) Boiss., *G. glabra* L. var. *glabra* is *G. echinata* L. The roots and rhizomes of the licorice plant and the licorice extract obtained from them are used. It contains licorice, starch, sugar (glucose, sucrose), gum, resin, bitter substance, flavone glycosides, glycyrrhizin, calcium, nitrogen, potassium and magnesium, asparagine and mannite. Glycyrrhizin is 50 times sweeter than sugar, its presence in roots varies between 5 - 13%. According to the analysis, it was

determined that there was 8.6% water, 5.5% ash, 31.9% extract (gum and starch), 1.5% glucose, 2.3% sucrose, 4.7% resin and 9.5% glycyrrhizin. has been. Licorice roots which find a wide variety of uses in the industry are used as an additive in the production of cola and used as an additive in the production of cola, and in the production of beer to foam. It is used as a taste modifier in the pharmaceutical industry, as well as in the preparation of tablets, and is also included in the composition of drugs used to soothe kidney and stomach diseases and nerves. It is mixed with tobacco to reduce the effect of nicotine in cigarette production. As it is used in the confectionery industry, licorice honey has also been used in the production of tahini halva in recent years. Press residues from the production of licorice honey are used in the production of wall plates called maftex. As a drug, it has phlegm and diuretic, reduces nicotine damages, cleans the bronchi, removes kidney diseases, reduces kidney and bladder stones, and heals ulcer wounds in the stomach (https: //www.kalkinmakutuphanesi.gov.tr /assets/ upload/ dosyalar/ adiyman- tibbi-ve-aromatik-bitkilerraporu_.pdf/ Date of access: 10.05.2021).

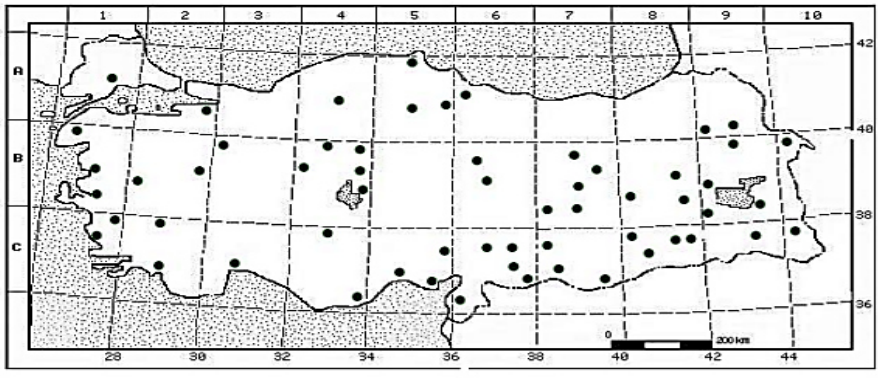


Figure 1. Spread Areas of Glycyrrhiza Glabra Species in Our Country (Çetin, 2015)

Licorice was mentioned as "super medicine" in Shen Nong Herbal, which was compiled about 2000 years ago in China and contains a list of more than 365 herbal medicines. Hayatizade Mustafa Feyzi Efendi, who was the chief physician of the sultan during the period of Sultan Mehmet the Fourth (1642-1693), described the root of Licorice. "It is beneficial for chest diseases, it removes the heat and thirst, the dried form is good for cataract disease if it is rubbed on the eyes, licorice root extract is good for chest pain, ulcers, bladder and kidney diseases, it is useful for cough" (http://e-kutuphane. teb.org.tr /pdf/ eczaciodasiyayinlari /ila_habr-eyll08/7.pdf/ Access Date:10.05.2021).

Glycyrrhiza species are used in ulcer treatment as antimutagenic, anti-arrhythmic, antimicrobial, antibacterial, anti-viral, anti-arthritic, anti-allergic, phlegm and anti-inflammatory (Çetin, 2015). The *Glycyrrhiza glabra* species has been used for medical purposes for about 4000 years. Humnubari laws contain records regarding the medical use of Glyycyrrhiza. Hippocrates mentioned that it is used in the treatment of ulcers and to quench thirst. Also mentioned as medicine in Theophrastus, Dioscorides, Pliny, Elder, Culpepper (Anagha et al., 2012). The Roman Union considered it an indispensable food in their long tiring expeditions. Roman soldiers said they could go without eating or drinking for 10 days, as it helped to energize and maintain stamina by quenching thirst and hunger (Anagha et al., 2012). *Glycyrrhiza glabra* is a plant that has been widely used both by the public and in the field of medical treatment since ancient times. This drug, which contains saponoside (glycyrrhizic acid) and flavonosides

30 (liquitoside and isolychritoside), which are still included in the composition of preparations used against ulcer and upper respiratory tract diseases by taking advantage of its antispasmotic, anti-inflammatory and antacid effects, is also a valuable export substance in terms of our country's economy. Glycyrrhiza species are given names such as Licorice, Miyan, Piyam, Payam, Payan in our country. In addition, in the Aegean Region, *Glycyrrhiza echinata* species are given names such as bitter pian, bitter root due to the bitter root. *Glycyrrhiza glabra* type is used in our country as a cold, cough, breast softener, preventing mucosal irritation and against ulcers (Tanker &Özkal, 1977-1978). The active ingredients of the genus Glycyrrhiza and their usability in drug production have been investigated by many researchers. Glycyrrhiza species contain saponin, flavonoid, polysaccharide, pectin, simple sugars, amino acids, mineral salts and some other substances (Kataria et al., 2013; Çetin, 2015).

Picture 1: Licorice Plant (https://www.kalkinmakutuphanesi.gov.tr/assets/upload/dosyalar/adiyman-tibbi-ve-aromatik-bitkiler-raporu_.pdf/ Erişim Tarihi:10.05.2021)

In this study, the colors, light and friction fastness values obtained by using various mordants from the licorice plant with yellow color scale feature were determined.

1.1. MATERIAL AND METHOD

The material of the study consists of the colors obtained by the dyeing method from the licorice plant, the fastness values and the use in textile fibers. Mordants used in the study were obtained from Sivas Cumhuriyet University Sivas Vocational School Handicraft Department, Painting Workshop. These mordants; 1.Aluminium alum - KAI (SO4) 2, 2. Copper sulphate (Eyebrow) - CuSO4.5H2O, 3. Iron sulphate (Cyprus) - FeSO4.7H2O, 4. Tartaric acid - (C2H2 (OH) 2 (COOH) 2-C4H6O6), 5. Acetic Acid, 6. Zinc Chloride, 7.Citric Acid, 8. Sodruy Hirdosulfite, 9. Copper II Sulphate, 10. Potassium Bi chromate-K2Cr2O7. In addition, mordant-free ropes were dyed and a comparison was made with the color absorption of mordant-free ropes. In the research, by scanning the sources about natural dyeing, dyeing and dressing methods, the mordant of the yarns, the preparation of the dye extract, the dyeing with and without mordant, the determination and naming of the colors obtained, the determination of light and friction fastness were stated.

As a method; Mordant of wool yarn, preparation of dye exracts, dyeing without mordant and mordant, determination and naming of colors, evaluation of colors, determination of light and friction fastness methods were used.

1.2. Mordant of Wool Yarns

Wool threads were mordant separately with each of the 10 different mordant materials specified in the material section. Mordant material was used at the rate of 2% and 4%, and wool yarn was dyed separately with each mordant. Mordant material is dissolved in 1 to 20 ratio of warm water, pre-moistened wool yarn is pressed into this mordant water. After boiling for one hour, the wool was allowed to cool in the boiling pan. After the ropes have cooled, they are squeezed out of excess water, dried and made ready for dyeing. At this stage, rinsing is never done.

1.3. Preparation of Hot Extract

The parts of the plants containing dyestuffs, dried fruit shells, all parts of the plant such as root-stem-branch-flower, stem shells, subsoil shoots were cut into small pieces by hand and knife in order to ensure that the dyestuffs they contain pass into the water. Later, the plants purchased at a rate of 100% according to the weight of the wool yarn to be dyed were boiled in water at a rate of 1 to 20 according to the wool to be dyed for 1 hour. At the end of 1 hour, the plant residues were removed from the environment by filtering with a cheesecloth. Thus, the hot extract was obtained.

1.4. Painting Process

The hot extract was obtained by using 100% of the plants. Previously standing in water for 1 hour soaked wool were put in the 20 to 1 ratio by weight extrakt. After reaching the boiling point, it was boiled for one hour with continuous stirring. Less water was added during boiling.

After cooling, it was rinsed with plenty of cold water and dried in a low light and airy place.

Picture 2: Boyama İşlemi (Kaynar, 2017)

In dyeing with mordant, the wools that were previously mordant were soaked in water for at least one hour before starting the dyeing process, and then boiled in a hot extract prepared at a rate of 1 to 20 for one hour and left to cool on their own. It was then rinsed with plenty of cold water and dried in an airy place with little light.

Picture 3: Drying Dyed Wool Yarns (Kaynar, 2017)

1.5. Determination and Naming of Obtained Colors

21 dyeings were done by applying the ratios of 2% and 4% with hot extracts obtained by using 100% of licorice plant without mordant and with different mordants. The colors obtained as a result of this painting were named by a commission.

1.6. Determination of Light Fastness and Friction Fastness

This stage belongs to the measurements of light and friction fastness, which are important for the use of dyed wool yarns in textile products. The determination of light fastness in dyed wool yarns was made on the basis of TS 867 (Color Fastness Determination Method against Daylight) (Anonymous, 1984a) and DIN 5033 (Farbmessung Begriffe der Farbmetrik) (Anonymous, 1970) methods prepared by the Turkish Standards Institute. For the determination of light fastness, blue wool scale (wool fabric strips dyed using various blue dyes graded from 1 to 8) and wool yarn samples were used. The blue wool scale is affixed on the cardboard from 1 to 8, respectively, 1 cm in length and 6 cm in width. Likewise, dyed wool yarn samples were wrapped parallel to each other, with a length of 1 cm and a width of 6 cm, on cardboard. 10 cm and 5 cm wide strips were cut from the cardboard, placed on top of each other and a binding was made. Wool yarn samples prepared in two parallel on cardboard cut in 7 cm width and blue wool scale samples cut in 1 cm width were placed on the cardboard skin in a way that half of it was closed while the other half could see daylight. After the samples were placed at 45 degrees to the incident of light, they were checked at the same times every day. Wool yarn samples were evaluated according

to the fading in the blue wool scale. The blue scale (blue dyed wool scale) is used only for light fastness measurement. Gray scale is used for all other fastnesses. In determination of friction fastness; Determination of friction fastness in dyed wool yarns according to TS 717 (Determination of Color Fastness to Friction) (Anonymous, 1978) prepared by the Turkish Standards Institute and TS 423 (Color Fastness Determination in Textile Products for the evaluation of stains (dye bleeding) and fading (color change) Using Methods of Gray Scales) (Anonymous, 1984b).

Dyed wool threads were wrapped side by side and parallel, 5 cm wide, on a 14 cm x 7 cm rectangular cardboard loop. By placing a dry, unpainted 5 cm x 5 cm sized plain textured cotton cloth on the tip of the test device, the dry samples prepared in two parallel under 900 gr load were rubbed back and forth 10 times in 10 seconds on a straight line along the 10 cm section. Color flow to unpainted cotton cloth was evaluated according to TS 423 with gray scale (Anonymous, 1984b).

2. FINDINGS

The values of the colors obtained in the study for light and friction fastness on wool carpet yarns are shown in Table 1. According to this; It was determined that the light fastness values of the colors obtained by using licorice plant and various mordants varied between (5--7), and the light fastness value of the color obtained by dyeing without mordant was found to be (5). In light fastness measurements, 4 and 5 are close to each other. Since 7 and 8 values are found in very few plants, 5 values

can be evaluated as (good) and 7 as (very good). It is seen that the light fastness value is quite high.

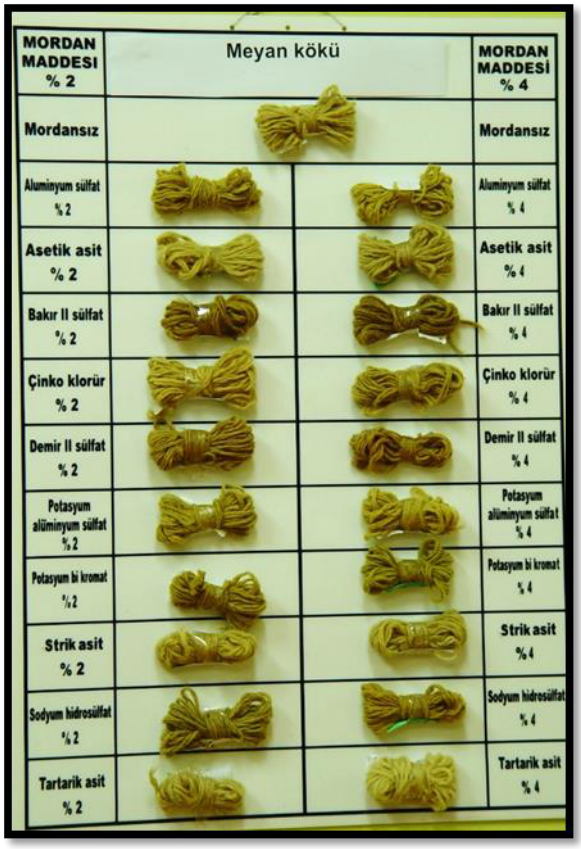
It is seen that the friction fastness values vary between (1-4) and the colors obtained by dyeing without mordant have friction fastness values (3-4). The lowest (1) value was found with Iron II Sulphate, and the highest value (4) was found in dyeing with Citric acid, Copper II Sulphate and Citric acid.

Table 1: Licorice Plant Light, Friction Fastnesses

SN	**Mordant**	**Light Fastness**	**Friction Fastness**	**Colors**
1	Acetic acid 2%	5	3_4	Olive oil green 1
2	Acetic acid 4%	5	3	
3	Copper II sulphate 2%	7	4	Olive oil green 2
4	Copper II sulphate 4%	7	3	
5	Zinc chloride 2%	5	2_3	Pickled Olives 2
6	Zinc chloride 4%	5	2_3	
7	Iron II sulphate 2%	7	1	Pickled Olives 2
8	Iron II sulphate 4%	7	1	
9	Potassium aluminum sulphate 2%	7	3	Olive oil green 3
10	Potassium aluminum sulphate 4%	7	3_4	Olive oil green 3
11	Potassium bi chromate 2%	5	4	Olive oil green 3
12	Potassium bi chromate 4%	5	2_3	Olive oil green 4
13	Citric acid 2%	7	3_4	Cumin 1
14	Citric acid 4%	7	4	Cumin 2
15	Sodium hydrosulfite 2%	5	4	Coffee foam 1
16	Sodium hydrosulfite 4%	5	3_4	Coffee foam 2
17	Tartaric acid 2%	5	3_4	Straw Yellow 1

18	Tartaric acid 4%	5	4_5	Straw yellow 2
19	Copper sulphate 2%	7	3	Pickled Olives 3
20	Copper sulphate 4%	7	2_3	Pickled Olives 4
21	Mordant free	5	3	Cumin2

Coloring samples made with licorice plant are given in Picture 4 and 5. The colors obtained are; Olive oil green, Pickled olive, cumin, straw yellow and coffee foam and coffee bean colors. The proportion of purple affects the color tone.



Picture 4: Licorice Color Chart -1 (Kaynar, 2017)

Picture 5: Licorice Color Chart -2 (Kaynar, 2017)

3. CONCLUSION

The negative consequences of rapid industrialization experienced today pandemicallay, terms of environment and human friendliness, has gained importance. Natural procedures that do not harm nature and people, use natural raw materials and do not leave chemical waste have started to be investigated again. The healing properties of plants against diseases have been known for thousands of years. Recently, there has been an awareness of what should be done to avoid getting sick before

treating a disease. In this context, to take protective precautions preventive f, it has become the priorities of developed countries. The usage areas of plants have also been expanded. In addition to treatment, natural substances and herbs have been used in preventive folk medicine.

It is known that the licorice plant, which has been used in the south and south-eastern provinces of our country for many years, has an important place among medicinal aromatic plants and is good for many diseases. In this study, dyeing experiments were carried out with licorice root in order to expand the usage areas of plants and to create an alternative to chemical substances. The results obtained have been evaluated in terms of the textile industry. When the results of the fastness tests are examined, it is seen that the light fastness which is an important feature for the dyes used in the textile industry, is at a good level. Different results were obtained in friction fastness. As a result, it has been determined that licorice root can be used as a dye in the textile sector by preventing dye erosion by natural methods.

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Picture 1: Licorice Plant (https://www. kalkinmakutuphanesi. gov.tr/assets/upload/dosyalar/adiyman-tibbi-ve-aromatik-bitkiler-raporu_.pdf/ Erişim Tarihi:10.05.2021)

Picture 2: Boyama İşlemi (Kaynar, 2017)

Picture 3: Drying Dyed Wool Yarns (Kaynar, 2017)

Picture 4: Licorice Color Chart -1 (Kaynar, 2017)

Picture 5: Licorice Color Chart -2 (Kaynar, 2017)

CHAPTER 16

GENERAL CHARACTERISTICS AND BIOLOGICAL ACTIVITIES OF RANUNCULUS SPECIES

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INTRODUCTION

People have entire time used plants for their requirements and also to treat illnesses (Huy et al., 2018; Du, 2018). Despite great advances in modern medicine, herbs make a great contribution to medicine. In this regard, about 11% of the fundamental drugs used are made up of plants and at the same time, most of the synthetic drugs are derived from natural components (Pandey et al., 2018). The growing interest in medicinal herbs is mainly due to the notion that natural products are more effective than synthetic products and also have fewer side effects. The fact that natural herbal medicines are more influential than synthetic medicines and have fewer side effects has increased the interest in medicinal herbs. Since plants are economically inexpensive, they are preferred as an alternative treatment, particularly in developing countries (Pandey et al., 2018). In addition to the curative use of herbs, it is also used in foods, beverages and cosmetics (Du, 2018).

Ranunculaceae (buttercup) family includes about 62 genera and 2200 species. Ranunculus belongs to the Ranunculaceae family and consists of about 600 species distributed worldwide (Emadzade et al., 2011; Hao, 2018). This genus can be seen on every landmass, from tropical regions to the Arctic and Subantarctic regions. It is denser especially in temperate regions and Mediterranean regions. Ranunculus plants live in a variety of environments including marshy land and cold alpine mountains. The Ranunculus genus has various morphological and physiological features and these features provide a strong adaptability (Hao, 2018). Turkey is represented by 94 native taxa, 82 of

which are at species level. These plants are annual or perennial and 19 of the taxa are indigenous to Turkey. All parts of Ranunculus plants are poisonous while alive. Toxins are destroyed when the plant is dried and heat is applied (Terzioğlu et al., 2008).

1. GENERAL CHARACTERISTICS OF RANUNCULUS SPECIES

1.1. Chemical Content

Various components have been isolated in Ranunculus species. In Ranunculus species, lactones such as protoanemonin, anemonine, ranunculin, isoranunculin and ternatolide show a wide distribution (Peng et al., 2006). The predominant volatile compound in Ranunculus is protoanemonin (Da-Cheng et al., 2015). Structurally ordinary alkaloids are usually found in Ranunculus and the main isoquinoline alkaloids are usually in the form of berberine and aporphine. It is known that whole saponins obtained from this genus are in the form of oleanan.. Ranunculus contains flavonoids such as apigenin, quercetin, luteolin, isoorientin, vitexin, tricin, orientin, saponaretin, gossypitrin and their glycosides (Hao et al., 2015).

1.2. Traditional Use

Many patient people in developing regions prefer traditional medicine (Mbuni et al., 2020). Traditional medicines are generally cheaper than modern medicines and are the only natural medicinal remedies available and accessible in remote rural areas (Popović et al., 2016). The Ranunculus genus has numerous conventional medicinal

species. Rhizomes, leaves and fruits of Ranunculus species are used medicinally (Hao, 2018). Traditionally, its most common use is for the treatment of rubella, antirheumatism and fever. For such uses, it is usually prepared by boiling the herb. At the same time, the healing properties of some Ranunculus species in conditions such as antihemorrhagic (Mantle et al., 2000), neuralgia pains, anti-spasmodic, diaphoretic (Leporatti & Ghedira, 2009), tympani, eye conjunctivitis, malaria, snake or scorpion venom, and acute icteric hepatitis (Pande et al., 2007) are available.

2. BIOLOGICAL ACTIVITIES OF RANUNCULUS SPECIES

Medicinal plants are a vital resource as they are used by humans in the treatment and prevention of diseases. Important bioactive compounds are extracted from plants (Mbuni et al., 2020). Bioactive plant metabolites have therapeutic value for the prevention and treatment of various cancers (Hao et al., 2015). Since plant phytochemicals have important bioactive properties for human health, they have been the focus of attention of researchers (Demir & Akpınar, 2020). Ranunculus species have various biological activities including various anti-cancer, anti-inflammatory, antioxidant, analgesic, antimicrobial and antiparasitic (Da-Cheng et al., 2015).

2.1. Anticancer Activity

Cancer is a global health problem. Side effects of the drugs used in traditional cancer treatment and the high cost of treatment cause limitations. Such restrictions have led to a search for new treatment

strategies. Therefore, herbs offer alternatives to create new, safe and powerful anticancer drugs through their bioactive components (Alami Merrouni & Elachouri, 2021). According to an in vitro study, *Ranunculus sieboldii* was found to have anticancer activity on four different human tumor cell lines (KB, BEL-7407, A549, HL-60) (Yun-xue et al., 2004). *Ranunculus ternatus* polysaccharides have been reported to induce apoptosis in MCF-7 cells and increase the activity of natural killer cells, thus inhibiting cancer cell growth (Sun et al., 2013). It has been demonstrated that ethyl acetate extract from *Ranunculus ternatus* exerts cytotoxic effects on human T cell lymphoma Jurkat cells. It has also been shown that cell death caused by ethyl acetate extract is due to caspase-7 (Fang et al., 2020). *Ranunculus constantinopolitanus* has been reported to have anticancer activity on the MDA-MB-231 breast cancer cell line (Taş et al., 2018).

2.2. Antioxidant Activity

The antioxidant abilities of plants provide the ability to scavenge harmful free radicals and prevent free radicals from damaging cells. This feature is mostly due to the antioxidant polyphenol content of the plants (Belščak-Cvitanović et al., 2018). The ethyl acetate fraction of the extract from *Ranunculus macrophyllus* roots was found to have strong radical scavenging and the ability to prevent peroxidation of lipids, and these activities were strongly associated with phenolic compounds (Deghima et al., 2020). *Ranunculus marginatus* has been reported to have antioxidant properties (Kaya et al., 2010). Methanol extract obtained from *Ranunculus arvensis* has been shown to exhibit

significant antioxidant activity (Bhatti, Ali et al. 2015). According to phytochemical studies on *Ranunculus muricatus*, it has been reported that a new lactone called muriolide has been isolated and has antioxidant activity (Raziq et al., 2020). *Ranunculus constantinopolitanus* has been reported to be a high antioxidant (Taş et al., 2018). It has been determined that ethyl acetate and n-butanol extracts obtained from *Ranunculus macrophyllus* show antioxidant activity (Deghima et al., 2021).

2.3. Anti-inflammatory activity

Inflammation is a biological response that protects the body from harmful factors, including pathogens. Inflammation prevents cell damage and provides regeneration of tissues and removal of necrotic tissues and cells (Fujiwara & Kobayashi, 2005). Various non-steroidal anti-inflammatory drugs are available that decrease pain and inflammation. Unfortunately, many side effects occur when these drugs are administered. However, herbs with little or no side effects and anti-inflammatory therapeutic effects can be used as an alternative (Oguntibeju, 2018). It has been demonstrated that *Ranunculus sceleratus* species has anti-inflammatory effects in vivo and in vitro studies. The non-polar extract inhibited eicosanoid synthesis (Prieto et al., 2003). Methanol extract of *Ranunculus pedatus* showed wound healing and anti-inflammatory effects (Akkol et al., 2012). It has been demonstrated that ethyl acetate and n-butanol extracts of *Ranunculus macrophyllus* show remarkable anti-inflammatory activity due to their high content of both phenolic compounds and triterpenoids (Deghima

et al., 2021). Methanol extract obtained from *Ranunculus bulumei* was found to have anti-inflammatory capacity by reducing nuclear factor kappa B (NF-κB) signal (Hong et al., 2020).

2.4. Antibacterial activity

Most medicinal plants produce compounds with antibacterial properties. These plants, with their high medicinal value, are widely used in society for the treatment of various diseases. It is known that indiscriminate use of antibiotics in the treatment of bacterial infections develops resistance. This has become a major clinical problem in the treatment of infectious diseases. In addition to this problem, many adverse conditions occur such as hypersensitivity to antibiotics, disruption of the intestinal flora, immunosuppression and allergic reactions. Consequently, the discovery and development of antimicrobial drugs may be alternatives for the treatment of many infectious diseases (Mirzaei, 2017). Accordingly, studies on antibacterial properties are carried out on various plants. For example, *Ranunculus marginatus* has been reported to have antibacterial activity (Kaya et al., 2010). It has also been reported that essential oils obtained from *Ranunculus constantinopolitanus* have antibacterial properties (Terzioğlu et al., 2008). Some components isolated from *Ranunculus laetus* species have been found to have antibacterial effects on *Escherichia coli, Bacillus subtilis*, *Salmonella typhi*, *Shigella flexinari*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Hussain et al., 2009). It has been investigated that *Ranunculus aestivalis* has active

antibacterial properties against *Klebsiella pneumoniae* and *Staphylococcus aureus* (Bonjar, 2004).

3. CONCLUSION

Ranunculus genus plants belonging to Ranunculaceae family show global distribution and many endemic species are located in Turkey. These plants have traditionally been used to treat a variety of ailments and are still used. These plants have been used traditionally in the treatment of various diseases and still continue to be used. Many chemical components have been isolated from Ranunculus species. These components have been shown to have a variety of biological activities. At the same time, these plants have various biological activities including various anticancer, anti-inflammatory, antioxidant, analgesic, antimicrobial, antiparasitic. Today, many restrictions in the treatment of diseases have led to the search for alternative treatment. Accordingly, further research on Ranunculus species may shed light on the treatment of diseases.

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