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Some Morphological and Chemical Characteristics of Sarsaparilla (*Smilax aspera* L., *Smilax excelsa* L.)

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Abstract

Two sarsaparilla species *Smilax aspera* L. and *Smilax excelsa* L. show natural distribution in Hatay flora. In the study, besides some phenological and morphological characteristics, antioxidant capacity and fixed oil contents and components of *Smilax* species collected from different locations were determined. The highest 100 fruit weight with 37.69 g was obtained from the sample of S. aspera L. species collected from Yayladagi 2 location and the highest 100 seed weight with 24.47 g from Yayladagi 1 location. In terms of antioxidant capacity of the leaves and fruits, insignificant differences were observed among the species and locations. The antioxidant capacities of leaves ranged 62.28 to 64.57 mmol.Fe+2/kg while fruit antioxidant capacities ranged from 63.91 to 66.31 mmol. Fe+2/kg. The highest value of seed fixed oil with 11.42% was obtained from the *S. aspera* L. sample collected in Yayladağı 3 location. Major fatty acid component was found as vaccenic acid in general for all samples. The highest content of vaccenic acid found as 37.50% from *S. aspera* seed samples of Yayladagi 2 location. As a result, the leaves and berries of smilax species could be considered as a significant natural antioxidant source.

Key words: *Smilax, antioxidant, fatty acids*

Saparna (*Smilax aspera* L., *Smilax excelsa* L.) Türlerinin Bazı Morfolojik ve Kimyasal Özellikleri

Özet

İki sarsaparilla türü, *Smilax aspera* L. ve *Smilax excelsa* L., Hatay florasında doğal olarak yayılış göstermektedir. Çalışmada, farklı lokasyonlardan toplanan bu türlerin bazı fenolojik ve morfolojik özelliklerinin yanı sıra, antioksidan kapasiteleri ve sabit yağ içerikleri ile bileşenleri belirlenmiştir.

Çalışmada, en yüksek 100 meyve ağırlığı 37.69 g ile Yayladağı 2 lokasyonundan, en yüksek 100 çekirdek ağırlığı 24.47 ile Yayladagi 1 lokasyonundan toplanan *S. aspera* L. türüne ait örneklerde elde edilmiştir. Yaprak ve meyvelerin antioksidan kapasitesi açısından, tür ve lokasyonlar arasında istatistiki olarak önemli bir farklılık görülmemiştir. Yaprakların antioksidan kapasiteleri 62.28 ile 64.57 mmol.Fe+2/kg arasında değişirken meyvelerin antioksidan kapasiteleri 63.91 ile 66.31 mmol.Fe+2/kg arasında değişmiştir. En yüksek çekirdek sabit yağ oranı %11.42 ile Yayladağı 3 lokasyonundan toplanan *S. aspera* L. türünde elde edilmiştir. Genel olarak sabit yağın ana bileşeni vaccenic asit olmuştur. En yüksek vaccenic asit oranı %37.50 ile Yayaladağı 2 lokasyonundan toplanan *S. aspera* L. türüne ait çekirdek örneklerinde belirlenmiştir. Sonuç olarak, *Smilax* türlerinin yaprakları ve meyveleri önemli bir doğal antioksidan kaynağı olarak değerlendirilebilir.

Anahtar kelimeler: *Smilax*, antioksidan, yağ asitleri

Introduction

Plants and animals, directly or indirectly, comprise human food resources. Rapidly growing world population, present nutritional problems and existing genetic resources are not sufficient to solve the problems. Therefore, the search for new plants with

nutritional and medicinal values has gained a great importance. Turkey is located in a very special position in terms of plant genetic diversity. As presented in the latest records, a total of 12 476 plant taxa lives in Turkey with 32.7% of endemism rate (Davis, 1988; Guner et al., 2000; Vural, 2003; Erik and Tarikahya, 2004; Ozhatay and Kultur, 2006; Ozhatay et al., 2009).

The genus *Smilax* (fam. Smilacaceae) in English called "greenbrier" or "sarsaparilla" is dioecious, perennial, deciduous/evergreen, wrapping, climber plant. Most of the *Smilax* colonies are spread by rhizomes, so only about one in three colonies have plants of both sexes. *Smilax* is a very damage-tolerant plant capable of growing back from its rhizomes after being cut down or burned down by fire. This, coupled with the fact that birds and other small animals spread the seeds over large areas, makes the plants very resilient. The berry of sarsaparilla is bright red to blue-black color, spherical shape, 5-10 mm in diameter, rubbery in texture and has a large, spherical seed in the center (Bailey, 1950; Busia, 2016; Davis, 1984). Sarsaparilla is traditionally used in the treatment of rheumatism, rheumatoid arthritis, stomach pain, bloating and skin diseases such as leprosy and psoriasis (Yeşilada et al., 1999; Foster and Duke, 2000; Van and Wink, 2004). These plants carry saponins at their roots and are used among the people because of the diuretic and sedative effect. Smilax species have attracted the attention of many researchers, many studies have been done to obtain biochemical, bioactive, morphological and germination aspects besides antioxidant capacity and nutritive value (Demo et al., 1998; D'Antuono and Lovato, 2003; Souri et al., 2004; Ozgul-Yucel, 2005; Longo and Vasapollo, 2006; Ozsoy et al., 2008; Salihoglu et al., 2010; Ivanova et al., 2011; Challinor et al., 2012; Delgado-Pelayo and Horneo-Mendez, 2012). Additionally, flavonoids, tannins and anthocyanins have been studied in the foliage and rhizomes of Smilax (Bruno et al., 1985; Longo and Vasapollo, 2006).

Smilax, which has 350-400 species worldwide, is presented only by *Smilax aspera* L. and *Smilax excelsa* L. species in Turkey. There are both *Smilax aspera* L. and *Smilax excelsa* L. species native to Hatay flora.

Smilax excelsa L. is one of the characteristic plants of Black Sea region distributed in Northern Anatolia, Thrace and Mediterranean sea coast. The species is named as 'Anatolian sarsaparilla', 'melocan', 'melvocan', 'silcan', 'diken otu', 'mamula', melevcen, 'siraca', 'kircan' and 'citirgi'in Turkish colloquial language. *Smilax excelsa* L. is a climber and a thorny plant that can grow up to 20 meters. The leaves are round or partly heart shaped, the edges of the leaves are spotless or small barbed. Plant grows in forests, shrubs and roadsides. Fruits have 3 seeds and red color (Baytop, 1984).The plant shoots are consumed as vegetables and also other parts of plants has been used in folk medicine (Baytop 1984, Asimgil 2003).

Smilax aspera L. which has a wide vegetation in the Mediterranean coast aslo called 'silcan', 'deli silcan', 'gıcır dikeni', 'kara silcan', 'sulcan' in Turkish colloquial language (Gurdal and Kultur, 2013). *S. aspera* L. is barbed, climber plant with a height up to 15 m. Leaves are arrows or spears, dense, short stalked, with sharp edges. The fruit is red or black when it matures to the size of a pea. In ethnobotany studies it is mentioned that *S. aspera* has a usage as a food and medicinal properties (Dogan et al., 2004; Ozsoy et al., 2008; Salihoglu et al., 2010; Gurdal and Kultur, 2013).

The aim of this study was to determine some morphological and phenological properties as well as antioxidant capacities and fixed oil content and components in *Smilax aspera* L. and *Smilax excelsa* L. species' from different locations of Hatay.

Materials and Methods

Materials

In the study, *Smilax aspera* L. plant samples were obtained from 4 different locations (Yayladagi 1, Yayladagi 2, Yayladagi 3 and Antakya) and *Smilax excelsa* L. plant samples were obtained from 6 different

locations (Defne, Iskenderun, Arsuz 1, Arsuz 2, Arsuz 3, Arsuz 4) in Hatay-Turkey. Altitudes ranged from 61 m to 905 m. Leaf and fruit samples were taken in August when fruits get matured. The species were identified by Assoc. Prof. Dr. Yelda GUZEL from University of Mustafa Kemal, Faculty of Science and Literature, Department of Biology.

a b

Figure 1. a-*Smilax excelsa* L. b-*Smilax aspera* L.

Methods

Morphological characteristics (leaf ratio (%), dry weight ratio (%), 100 fruit weight (g), 100 seed weight (g), seed ratio (%)) and antioxidant analysis (leaf and fruit (mmol Fe+2/kg) and the fixed oil contents and components were analyzed. All the measurements were done with 3 replications.

Morphological characteristics

Leaf ratio (%): Leaves and stems were separated, weighed and the ratio was calculated for each plant sample. Dry weight ratio (%): The samples was weighed before and after drying on 35 oC. 100 fruit weight (g): 100 randomly chosen fruits which were weighed four times. 100 seed weight(g): After the seeds were removed from fruits, they were dried for 12 hours on a room temperature, than they weighed. Seed ratio (%): Fruits and seeds were weighed and the proportion of seed weight was calculated as % to fruit weight. Fruit samples could not be taken only in Arsuz district, so analysis on fruit could not be done.

Antioxidant capacity

Leaf and fruit antioxidant capacity (mmol Fe+2/kg): Antioxidant capacity of the samples were analyzed with FRAP (The Ferric Reducing Ability of Plasma) method from Pellegrini et al. (2003). Antioxidant capacity of samples was measured with spectrophotometer. Absorbance values were calculated from the curve factor obtained from $FeSO_4$ x $7H_2O$ (100 µmol/L) and the results were presented as mmol Fe^{+2}/kg (dry weight).

Seed fixed oil contents and components

The seeds were dried, grounded and extracted in soxhlet apparatus with hexan. Oil samples were kept in dark bottles at 4°C until chemical analysis were performed. Esterification: 1.5 ml 2N methanolic potassium hydroxide were added on 60 µl oil sample then vigorously shacked. 4 ml n-

heptane were added in this solution and shacked 30 second. The samples were kept 10 minutes for phase distinction and 1.5 ml of the upper phase was taken for analysis. The fatty acid composition of esterified seed oils was determined by GC/MS (Hewlett Packard Model, 6890/5972). Capillary column film thickness was DB-23 of 60 m length × 0.25 mm i.d. and 0.25 µm. The carrier gas was helium at 1.0 ml/min ratio. The injection volume was 1 µL. Fatty acid components were identified by comparing their retention times with those of reference compounds. MS transfer line temperature was 250 °C, MS ionization temperature was 220 °C, colon temperature was 120°C at the beginning 3 min holding at this temperature, has risen up 180 °C with 10°C/min, holding 10 minutes at 180 °C, risen up to 250 °C with 10°C/min, holding 19 minutes at 250°C. The total duration of the analysis was 45 minutes per sample.

Results and Discussion

In the study, it was determined that both Smilax species from different locations in Hatay region flowered in March, began to produce fruit in July and began to mature in August.

Morphological characteristics

Morphological characteristics of the genotypes are given in Table 1. Leaf and shoot ratio results varied between 30.95-65.35%. The highest ratio was obtained from Arsuz 4 location and the lowest ratio was obtained from Arsuz 1 location in *S. excelsa* L. Dry leaf ratio ranged between 40.81% (Yayladagı 3 location) and 65.42% (Antakya location) in *S. aspera* L. Temel and Tan (2011) reported that the leaf and shoot ratio ranged between 20.00-33.70%, while dry leaf ratio ranged as 16.6-36.5%. This difference could depend on different altitudes and abiotic factors. In terms of 100 fruit weight, there were big variations among the genotypes and the highest values were obtained from Yayladagı 2 location with 37.69 g in *S. aspera* species. The smallest fruits with 7.32 g were found in Arsuz 2 location in S. excelsa species. The highest 100 seed weight with 6.12 g was obtained from Yayladagı 1 location in *S. aspera* species and the lowest value with 3.83 g from Antakya location in *S. aspera* species. Ozgul-Yucel (2005), reported that 100 seed weight of *S. aspera* L. species are 6.0 g which are similar to our results. The highest seed ratio value with 32.0% was obtained from Iskenderun location in *S. excelsa* and the lowest value with 22% from Defne location in *S. aspera* L. Ozgul-Yucel (2005) reported that *S. aspera* L.collected from İstanbul had seed ratio with 43.4% higher than smilax species grown in Hatay flora. This difference might be due to climatic conditions or genotypes.

Leaf and fruit antioxidant capacity

Leaf and fruit antioxidant capacity of *Smilax* species are given in Table 2. Analysis showed very similar results in species, locations and plant parts. The highest antioxidant capacity with 66.31 mmol.Fe+2/kg was found in fruit samples of *S. aspera* L. in Antakya location, while the lowest value with 62.28 mmol.Fe+2/kg found in *S. excelsa* L. leaf samples collected from Arsuz 2 location. The results showed that two *Smilax* species have high antioxidant capacity. Antioxidant capacity of *S. excelsa* L. was studied by Ozsoy et al., (2008) using different methods (linoleic acid system and β-carotene bleaching method) and found that the antioxidant capacity was similarly high as these results.

Table 1. Morphological features of different Smilax genotypes

Locations	Species	Leaf-shoot ratio (%)	Dry leaf ratio (%)	100 fruit weight (g)	100 seed weight (g)	Seed ratio (%)
Yayladagi 1	*S. aspera* L.	45.19	52.58	35.84	6.12	28
Yayladagi 2	*S. aspera* L.	46.82	50.00	37.69	5.70	25
Yayladagi 3	*S. aspera* L.	60.27	40.81	33.16	5.81	26
Antakya	*S. aspera* L.	33.13	65.42	24.78	3.83	26
Defne	*S. excelsa* L.	31.06	47.33	25.57	3.91	22
Iskenderun	*S. excelsa* L.	60.00	61.27	21.74	4.14	32
Arsuz 4	*S. excelsa* L.	65.35	50.50	•	•	•
Arsuz 1	*S. excelsa* L.	30.95	53.43	•	•	•
Arsuz 2	*S. excelsa* L.	45.13	50.59	•	•	•
Arsuz 3	*S. excelsa* L.	32.60	58.02	•	•	•

• Fruit could not be taken

Table 2. Leaf and fruit antioxidant capacity of Smilax genotypes (mmol.Fe^{+2}/kg)

Locations	Species	Leaf antioxidant capacity	Fruit antioxidant capacity
Defne	*S. excelsa* L.	62.48	65.71
Yayladagi 1	*S. aspera* L.	64.57	65.71
Yayladagi 2	*S. aspera* L.	63.24	65.11
Yayladagi 3	*S. aspera* L.	62.97	64.51
Antakya	*S. aspera* L.	63.90	66.31
Iskenderun	*S. excelsa* L.	64.07	63.91
Arsuz 4	*S. excelsa* L.	62.53	•
Arsuz 1	*S. excelsa* L.	62.36	•
Arsuz 2	*S. excelsa* L.	62.28	•
Arsuz 3	*S. excelsa* L.	62.43	•

• Low amount of sample

Table 3. Seed oil ratios and fatty acids compositions of Smilax species from different locations

Locations	Seed oil ratio (%)	Lauric acid C12:0	Myristic acid C14:0	Palmitic acid C16:0	Stearic acid C18:0	Vacceni c acid C18:1	Linoleic acid C18:2	Gondoic acid C20:0
Yayladagi 1 (*S. aspera*)	11.18	4.21	13.39	12.67	3.75	33.14	31.34	----
Yayladagi 2 (*S. aspera*)	11.28	8.47	22.33	15.07	5.72	37.50	8.75	----
Yayladagi 3 (*S. aspera*)	11.42	5.88	16.06	13.10	3.59	30.93	27.44	2.30
Defne (*S. excelsa*)	10.15	10.23	26.93	20.41	5.72	31.97	----	----
Iskenderun (*S. excelsa*)	10.72	5.40	13.60	11.72	4.00	32.69	29.27	2.63

Seed fixed oil contents and components

Seed fixed oil contents and components are given in Table 3 and examples of chromatograms are given in Figure 2 and 3. Fixed oil contents of seeds ranged between 10.15% (Defne location in *S. excelsa* L.) and 11.42% (Yayladağı 3 location in *S. aspera* L.). Fatty acid components of the species showed large variations among the locations. Smilax genotypes contain both saturated (lauric acid, myristik acid, palmitic acid, stearic acid) and unsaturated (vaccenic acid, linoleic acid and gondoic acid) fatty acids. As we looked through the results for *S. aspera* L. genotypes; Yayladağı 1 location vaccenic acid and linoleic acid are found as main components with the amounts of 33.14% and 31.34% respectively. Lauric acid and stearic acid were found in low ratio as 4.21% and

3.75%, respectively. Fatty acid compositions of Yayladagi 2 location, from highest to lowest ratio, were found as vaccenic acid (37.50%), myristic acid (22.33%), palmitic acid (15.07%), linoleic acid (8.75%), lauric acid (8.47%) and stearic acid (5.72%). Seeds fatty acid components from Yayladağı 3 locations, from highest to lowest percentage, were found as vaccenic acid (30.93%), linoleic acid (27.44%), myristic acid (16.06%), palmitic acid (13.10%), lauric acid (5.88%), stearic acid (3.59%) and gondoic acid (2.30%). These results showed similarities with the result that Ozgul-Yucel (2005) reported.

Seed fatty acid composition of *Smilax excelsa* L., collected from Defne location, varied from highest to lowest, as follows vaccenic acid (31.97%), myristic acid (26.93%), palmitic acid (20.41%), lauric acid (10.23%) and stearic acid (5.72%). Seed fatty acid composition found as vaccenic acid (32.69%), linoleic acid (29.27%), myristic acid (13.60%), palmitic acid (11.72%), lauric acid (5.40%) and stearic acid (4.00%) in Iskenderun location.

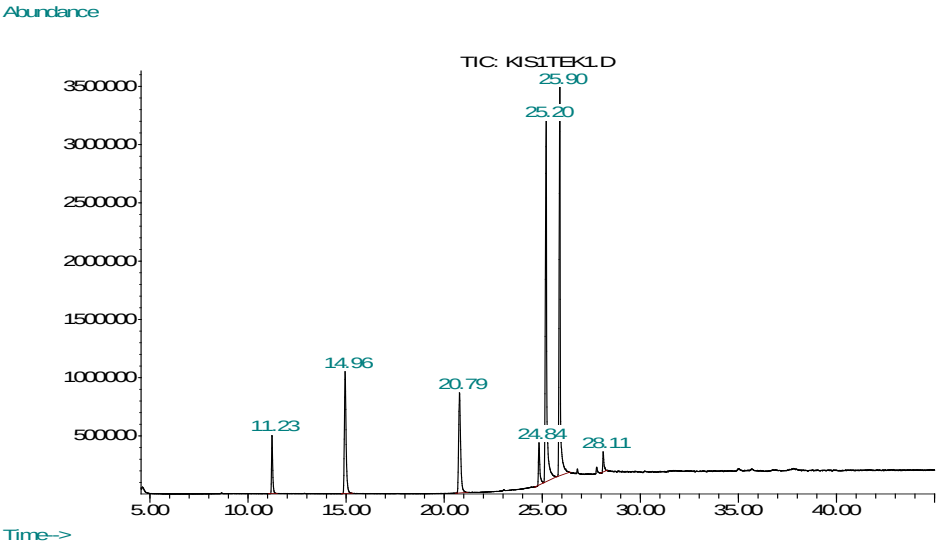

Figure 2. Chromotogram of *S. aspera* collected from Defne location

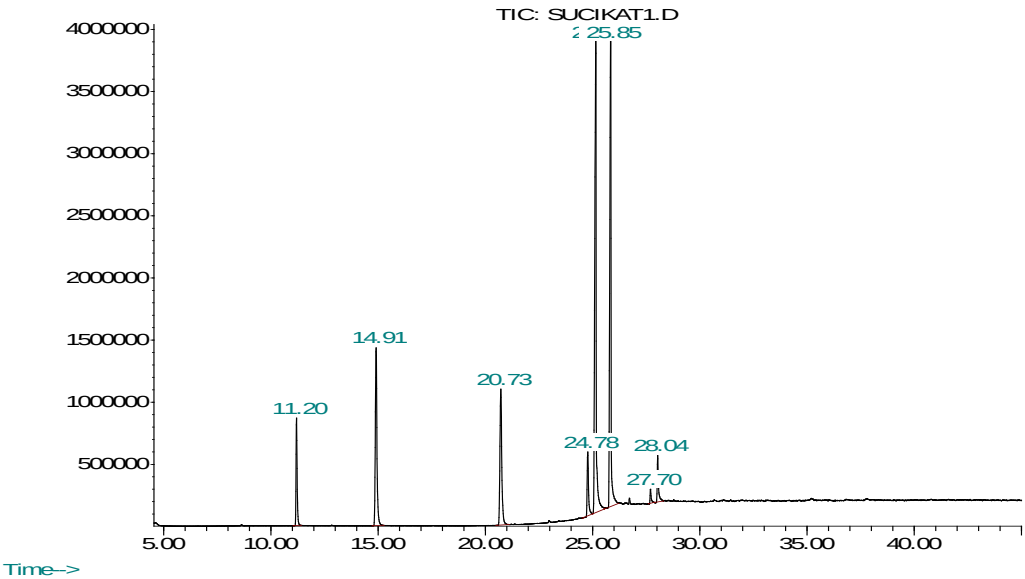

Figure 3. Chromotogram of *S. excelsa* collected from İskenderun location

The results showed that Smilax species have rich antioxidant capacity and it can be concluded that, the leaves and berries of *Smilax* species could be considered as a significant natural antioxidant source and can be used as an accessible source of natural antioxidants with consequent health benefits. These species are also rich in unsaturated fatty acids.

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