DETERMINATION OF SOME MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS OF SARSAPARILLA (*Smilax aspera* L. and *Smilax excelsa* L.)

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Abstract
Two sarsaparilla species *Smilax aspera* L. and *Smilax excelsa* L. shows 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. In the study, the highest 100 fruit weights with 37.69 g were 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, nonsignificant differences were observed among the species and locations. The antioxidant capacities of leaves were changed 62.28 to 64.57 mmol.Fe^{+2}/kg while fruit antioxidant capacities were ranged 63.91 to 66.31 mmol. Fe^{+2}/kg. The highest value of seed fixed oil with 12.03% were obtained from the *S. aspera* L. sample collected in Iskenderun location. Major fatty acid component were 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.

Keywords: Antioxidant, fixed oil, fatty acids, GC-MS.

Introduction
Plants and animals are 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 nourishment and medicinal facilities has gained a great importance. Turkey located in a very special position in terms of plant genetic diversity. The result of the new records, total of 12 476 plant taxon lives in Turkey with 32.7% endemism rate (Davis et al., 1988; Guner et al., 2000; Vural, 2003; Erik and Tarikahya, 2004; Ozhatay and Kultur, 2006; Ozhatay et al., 2009).
The genus *Smilax* L. (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 hard to get rid of. The berry of sarsaparilla is bright red to blue-black spherical color, 5-10 mm in diameter, rubbery in texture and has a large, spherical seed in center. Sarsaparilla is traditionally used in the treatment of rheumatism, rheumatoid arthritis, stomach pain, bloating and skin diseases such as leprosy and psoriasis (Yesilada et al., 1999; Foster et al., 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, has only *Smilax aspera* L. and *Smilax excelsa* L. species in Turkey. Hatay is in the south of Turkey, Mediterranean in the west, Syria in the south and east, Adana in the northwest, Osmaniye in the north and Gaziantep in the north. There are more than 2000 species found in the region, which about 300 are endemic and 400 are medicinal and aromatic plants. There are *Smilax aspera* L. and *Smilax excelsa* L. species in Hatay flora. *Smilax excelsa* L. is one of the characteristic plants of Black Sea region distributed in Northern Anatolia, Thrace, some provinces of Western Mediterranean coast and in coast east only Hatay province. The specie is named ‘Anatolian sarsaparilla’, ‘melocan’, ‘melvocan’, ‘silcan’, ‘diken out’, ‘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 part 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 shoots of plant are consumed as vegetables and also other parts of plants has been used in folk medicine (Baytop 1984, Asimgil 2003). Smilax aspera which has a wide vegetation in the Mediterranean coast aslo called ‘silcan’, ‘deli silcan’, ‘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 presents S. aspera has a usage as a food and medicinal properties (Dogan et al., 2004; Ozsoy et al., 2008; Raul and Damaso, 2012; Salihoglu et al., 2010; Gurdal and Kultur, 2013).

Figure 1. *Smilax excelsa* L.

Figure 2. *Smilax aspera* L.

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

Material and methods

Material

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 were ranged from 61 m to 905 m. The species were identified by Asst. Prof. Yelda GUZEL from University of Mustafa Kemal, Faculty of Science and Literature, Department of Biology.

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)) were done in Field Department Laboratories, the fixed oil contents and components were analyzed in Mustafa Kemal University Research and Application Center for Technology and Development. All the measurments were done 3 replicates.

Morphological characteristics

Leaf ratio (%): Leaves and stems were separated, weighed and ratio were calculated for each plant samples. Dry weight ratio (%): Fresh samples weighed after the samples got dried in 35 ^{o}C, weighed again and the ratio calculated. 100

fruit weight (g): 100 fruits for four times which randomly chosen were weighed. 100 seed weight(g): After the seeds removed from fruits, seeds dried 12 hours in room temperature then dry seeds weighed. Seed ratio (%): Fruits and seeds were weighed and the proportion of seed weight to fruit weight was calculated as%.

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). According to this method, 250 ml buffer acetate, 25 ml TPTZ (2,4,6-tri-2-pyridyl-s-triazin) and 20 ml $FeCI_3x6H_2O$ mixture FRAP reagent solution were prepared. Finally 900 µl distilled water, 300 µl extracted sample solution and 9000 µl FRAP reagent solution were mixtured. This mixture were then left at 37 °C for 10 minutes. Finally antioxidant capacity of samples were measured with spectrophotometer. Absorbance values were calculated from the curve factor obtained from FeSO4 x 7H2O (10-100 µmol/L) and the results are presented as mmol Fe^{+2}/kg (dry weight).

Seed fixed oil contents and components

After the seeds were grounded, samples left in oven for to loose moisture then samples were extracted in soxhlet apparatus with hexan. Oil samples were kept in dark bottles at 4 °C until chemical analysis. Esterification: 1.5 ml 2 N methanolic potassium hydroxide were added on 60 µl oil sample then vigorously shaked. 4 ml n-heptane were added in this solution and shaked 30 second. Sample were kept 10 minutes for phase distinction than 1.5 ml of the upper phase were taken for analysis. After seed oils were esterified fatty acid composition were determined with GC/MS (Hewlett Packard Model, 6890/5972) apparatus. 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, totally analysis run for 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 genotypes are given in Table 1. Leaf and shoot ratio results varied between 30.95-65.35%. Highest ratio were obtained from Arsuz

4 and lowest ratio were obtained from Arsuz 1 location. Dry leaf ratio ranged between 40.81% (Yayladagı 3 location) and 65.42% (Antakya location). 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 be depend on different altitudes and abiotic factors. In terms of 100 fruit weight, there were big variations among the genotypes and the largest fruits with 37.69 g were harvested from Yayladagı 2 location in *S. aspera* species and the smallest fruits with 7.32 g from 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 from Antakya location in *S. aspera* species. Ozgul-Yucel (2005), studied *Smilax aspera* L. 100 seed weight and found as 6.0 g similar to our results. The highest seed ratio value with 32.0% were obtained from Iskenderun in *S. excelsa* and lowest value with 22% from Defne location in n *S. aspera*. Ozgul-Yucel. (2005) reported that *S. aspera* collected from İstanbul had highest seed ratio with 43.4% than smilax species grown in Hatay flora. This difference might be due to climatic conditions or genotypes.

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	12.16	•	•
Arsuz 1	*S. excelsa* L.	30.95	53.43	8.10	•	•
Arsuz 2	*S. excelsa* L.	45.13	50.59	7.32	•	•
Arsuz 3	*S. excelsa* L.	32.60	58.02	8.52	•	•

• Low amount of sample

Leaf and fruit antioxidant capacity

Leaf and fruit antioxidant capacity of Smilax species were given in Table 2. Analysis showed very similar results in species, locations and plant parts. Highest antioxidant capacity found from Antakya fruit samples as 66.312 mmol.Fe^{+2}/kg while lowest found in Arsuz 2 location as 62.282 mmol.Fe^{+2}/kg. The results showed that two Smilax species have high antioxidant capacity. Antioxidant capacity of *S. excelsa* were obtained in previous study from Ozsoy et al. (2008) BHA, β-carotene and DPPH method in water extract, infusion, ethanol extract and ethyl acetate extract. However it is impossible to compare results from different methods.

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

Seed fixed oil contents and components

Seed fixed oil contents and components were given in Table 3, some examples of GC\MS were given in Figure 3 and 4. Fixed oil contents of seeds were ranged between 10.15-12.03%. Highest amount found in Iskenderun location, while the lowest found in Yayladagi 3 location. Fatty acid components from GC/MS analysis showed very variations in locations for main components.

Fatty acids can be divided in two sub group saturated fatty acids and unsaturated fatty acids. Seed oil analysis showed that Smilax genotypes contain both saturated and unsaturated fatty acids. When fatty acids enter the body they transform and has many features as antiviral, antimicrobial, antiprotozoal and antifungal for human body. One of the most important unsaturated fatty acid called essential fatty acids, that humans and other animals cannot synthesize them however they must ingest because the body requires them for good health, linoleic acid is one of them. *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 look through the results for *S. aspera* genotypes; Yayladagi 1 location (S. aspera) vaccenic acid and linoleic acid found as main components with the amount of 33.14% and 31.34% respectively. Lauric acid and stearic acid were found low ratio as 4.21% and 3.75%, respectively. Fatty acid compositions of Yayladagi 2 (*S. aspera*) 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 Yayladagi 3 (*S. aspera*) locations' from highest to lowest 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%). Duzici (*S. aspera*) locations' fatty acid components from highest amount to lowest amount were found as myristic acid (30.98%), palmitic acid (27.02%), vaccenic

acid (18.19%), lauric acid (10.84%) and strearik acid (8.89%). These results showed similarities with the result that Ozgul-Yucel (2005) reported..
Smilax excelsa seed samples could be taken from 2 loactions. Defne locations' seed fatty acid composition 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%). Iskenderun locations' seed fatty 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%).

Table 3. Fixed oil contents and components 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	Vaccenic acid C18:1	Linoleic acid C18:2	Gondoic acid C20:0
Antakya (*S. aspera* L.)	12.03	10.84	30.98	27.02	8.89	18.19	----	----
Yayladagi 1 (*S. aspera* L.)	11.18	4.21	13.39	12.67	3.75	33.14	31.34	----
Yayladagi 2 (*S. aspera* L.)	11.28	8.47	22.33	15.07	5.72	37.50	8.75	----
Yayladagi 3 (*S. aspera* L.)	11.42	5.88	16.06	13.10	3.59	30.93	27.44	2.30
Defne (*S. excelsa* L.)	10.15	10.23	26.93	20.41	5.72	31.97	----	----
Iskenderun (*S. excelsa* L.)	10.72	5.40	13.60	11.72	4.00	32.69	29.27	2.63

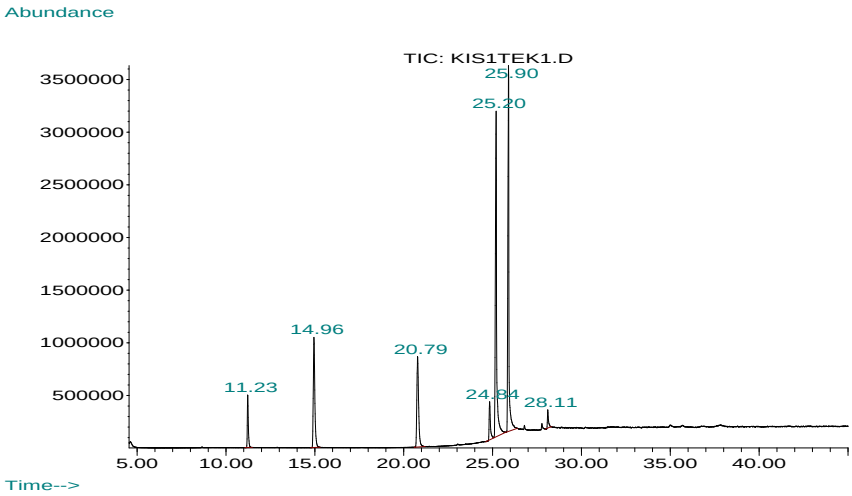

Figure 3. Chromotogram of Defne location (*S. aspera*)

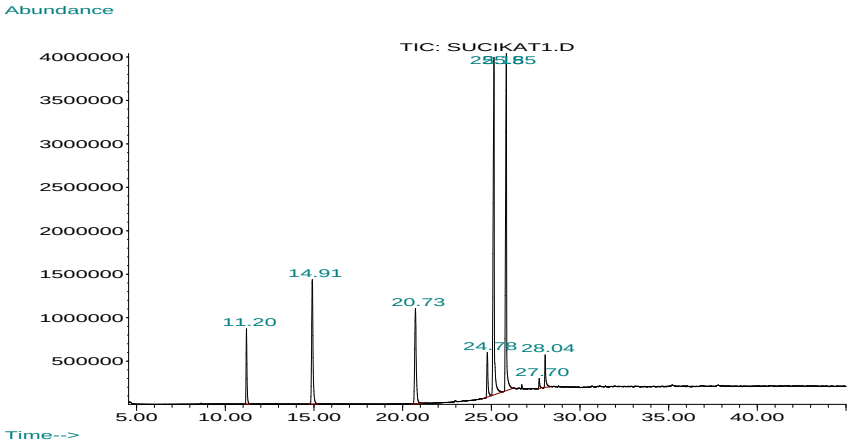

Figure 4. Chromatogram of Iskenderun location (*S. excelsa*)

Conclusions

The results showed that *Smilax* species have rich antioxidant capacity and 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 have also rich in both saturated and unsaturated fatty acids. All of these makes *Smilax* can be useful in all kinds of industries.

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