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Amount of Vitamin A, Vitamin E, Vitamin C, Malondialdehyde, Glutathione, Ghrelin, Beta-Carotene, Lycopene in Fruits of Hawthorn, Midland (*Crataegus laevigata*)

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Abstract

In this study, the amounts of vitamin A, vitamin E, vitamin C, β-carotene, lycopene, ghrelin, reduced and oxidized form of glutathione (GSH, GSSG) with malondialdehyde (MDA) in fresh fruits of *Crataegus laevigata* plant were determined by using High Performance Liquid Chromatography. The amount of vitamin A, vitamin E, vitamin C, β-carotene, lycopene, ghrelin, GSH, GSSG and MDA in fresh fruits of *Crataegus laevigata* plant were found to be 0.76 ± 0.08 - 1.14 ± 0.11 µg/g; 0.83 ± 0.10 - 1.17 ± 0.12µg/g; 16.45 ± 1.31 - 50.76 ± 2.02µg/g; 2.88 ± 0.24 - 3.87 ± 0.38 µg/g; 0.44 ± 0.06 - 2.34 ± 0.18 µg/g; 18.96 ± 6.73 - 79.96 ± 12.14 µg/g; 82.25 ± 17.26 - 564.88 ± 81.53µg/g; 118.02 ± 22.39 - 307.79 ± 52.50 µg/g and 24.27 ± 2.81 - 30.69 ± 6.83µg/g, respectively. It can be said that *Crataegus laevigata* plant fruits are good source of vitamin C, β-carotene, lycopene, ghrelin and glutathione. Generally, *Crataegus laevigata* is planted as an ornamental plant but these findings suggested that the fruit of this plant might be useful source of antioxidant. The differences in the amount of parameters in the *Crataegus laevigata* fruit are thought to be caused by the growth medium, climate and environmental conditions.

INTRODUCTION

Crataegus laevigata, is known as the mid land hawthorn, English hawthorn, wood land hawthorn or May flower, which is a species of hawthorn native to western and central Europe. It is also present in North Africa. The species name is sometimes spelled *C. levigata*, but the original orthography is *C. levigata* [1]. *Crataegus* species form a well-defined genus known as Hawthorns which belongs to the tribe *Crataegus* and sub family *Maloideae* that constitute a large number of about 28 genera and 940 species that are of economically and ecologically important trees and shrubs [2]. Hawthorn is an extremely valuable medicinal herb. It is used mainly for treating disorders of the heart and circulatory system, especially angina. Western herbalists consider it a "food for the heart". It increases the blood flow to the heart muscles and restores normal heartbeat [3]. Its fruit contains bioflavnoids which have high antioxidant properties and prevent deterioration of the blood vessels [4]. The fruit has been found to be cardio protective, antispasmodic, diuretic, sedative, tonic and vasodilator [5]. Hawthorn extracts have a long history of use in Europe for treating congestive heart failure, especially when combined with digitalis or other plants containing cardioactive glycosides and arterosclerosis [6]. Hawthorn fruits and leaves have abundant content of flavonoids and procyanidins which have antioxidant free radical scavenging, anti-inflammatory, vasorelaxing and hypolipidemic properties [7,8]. Free radicals that are produced by normal reductive biosynthetic pathways and also those that are produced as a result of many disease conditions leads to cell damage if not neutralized [9,10]. As a result of this, there is an increasing interest in natural antioxidants present in medicinal plants and dietary fruits that may help prevent oxidative damage [11,12]. *Crataegus* species have been used since ancient times, and the antioxidant constituents of the plant may account for its therapeutic use all over the world. Several hawthorn species are also used as folk medicine [13,14]. Recently, a lot of experimental work was conducted to determine the antioxidant activities of *Crataegus* species [15,16]. Some of the experiments were based on the antioxidant capacity of the whole plant [17], leaves [18], while some on the fruits of the *Crataegus* species [11,19]. Although Turkey is one of the genetic centers of *Crataegus* [20], however, few studies have attempted to describe the *Crataegus* strains in Turkey. Generally, *Crataegus* species are used for health treatment in Turkey [21].

This present research, compare the content of some important parameters (vitamins A, C, E, lycopene, beta-carotene, ghrelin, malondialdehyde, reduced and oxidized glutathione) in *Crataegus laevigata* fresh fruits from three different regions of Turkey (Elazig, Malatya and Adiyaman).

MATERIALS AND METHODS

Materials

Ripe *Crataegus laevigata* harvested from Elazig, Malatya and Adiyaman regions of Eastern Anatolia province of Turkey were used for the study. Samples were collected between 20-25th of November, 2016. Samples collected were identified by Dr. Şemsettin CİVELEK faculty member, Biology department Firat University (Herbarium No = 1810).

Determination of vitamin A, vitamin E, β-carotene and lycopene

1.0 g from each *Crataegus laevigata* sample was weighed and 5.0mL methyl alcohol was added. The suspension was shaken well in a vortex mixer and then centrifuged for three minutes at 4500 rpm. The solution was filtered (Whatman No 1). 0.2 mL n-hexane was added to the filtrate. This way, vitamin A, vitamin E, β-carotene and lycopene were extracted to the n-hexane phase. This extraction process was replicated. The n-hexane phases were collected and dried with nitrogen gas until dryness. There residue obtained was dissolved in 0.2 mLmethylalcohol, which was then ready for HPLC analysis. An ODS-2 column (25cm, 4.6 mm ID, 5 µm) was used for the determination of vitamins A, E, β-carotene and lycopene. Methanol-Acetonitrile-Chloroform mixture (47:42:11 v/v) was used as mobile phase at 1.0 mL/min flow rate. HPLC peaks were measured at 296 nm for vitamin E, 326 nm for vitamin A, 436 nm for β-carotene and at 460 nm for lycopene [22,23].

Determination of vitamin C, ghrelin, glutathione and MDA

A 1.5 g from each *Crataegus laevigata* sample was weighed and 1.0mL of 0.5 M perchloric acid was added to each added to the homogenate, to precipitate the proteins. The mixture was placed in a vortex shaker. Each sample was made up to 5.0 mL with deionized water and centrifuged for 10 minutes at 4500 rpm. The supernatant was then filtered (Whatman No 1). The filtrate was divided into three equal portions. One portion was used for vitamin C while the others were used for ghrelin, glutathione and MDA determination. A 20 µL of the supernatant was directly injected to HPLC. Vitamin C, Ghrelin, GSH, GSSG and MDA were determined according to method Tavazi et al. (1992) [24], Aydin et al. (2008) [25], Dawes and Dawes (2000) [26], and proposed by Karatas et al. (2002) [27], respectively. Column Exsil 100-5 ODS (25 cm, 4.6 mm ID, 5 µm) flow rate of 1mL/min. The calibrationcurves and thelinear equation derived from the calibration curve for each parameter are given in Figure 1.

Equipment and chemicals

HPLC was performed with the SHIMADZU Prominence-İ LC-2030C 3D Modeland PDA detector. The All the chemical reagents used in the analysis were analytical grade and obtained from Merck (Darmstadt, Germany). Double distilled water was used through out the work.

Statistical analysis

All measurements were triplicated. Mean standard deviation was determined and the results were subjected to Analysis of Variance. The SPSS 10.0 for Windows was used for variance analysis and LSD multiple comparison test was performed at $p<0.05$ to $p<0.005$ level.

RESULTS AND DISCUSSION

Table 1 some parameters in *Crataegus laevigata* specimens from three different provinces Oxidation plays an important role in the development of many diseases. The consumption of antioxidant-rich nutrients that inhibit oxidation is very important for metabolism. Vitamin A plays a role in increasing the body's resistance to infections, bone growth, visual functions, skin development, reproduction, cell division and, as well as strengthening the immune system [28]. The result obtained in this study showed the amount of vitamin A in *C. laevigata* from Elazig, Adiyaman and Malatya to be 0.78 ± 0.08 µg/g, 0.76 ± 0.08 µg/g and 1.14 ± 0.11 µg/g respectively. With that of Malatya having a higher concentration of vitamin A, followed by that of Elazig, then Adiyaman (Table 1).

Also the vitamin A content of uvaia (*Eugenia pyriformis*) was found to be 37.83 µg/g [29]. Based on the results obtained in this study, the vitamin A content of *C. laevigata* is higher than that of *Pyracantha coccinea*, red cherry and strawberry but much lower than that of uvaia (*Eugenia pyriformis* Cambess).

Vitamin E protects the unsaturated fatty acids in the cell membrane against the oxidation of free radicals. It stops this reaction (which is the initiator of autooxidation) by saturating peroxide and hydroperoxides with hydrogen ions, reducing the activity of peroxide radicals [28,30]. This study showed the vitamin E content of *C. laevigat*a from Elazig, Adiyaman and Malatya to be 0.83 ± 0.10µg/g,1.17 ± 0.12µg/g and 0.89 ± 0.09µg/g, respectively. Based on these values the vitamin E content of *C. laevigata* from Adiyaman (1.17 ± 0.12µg/g.), is higher than that of Malatya (0.89 ± 0.09 µg/g) and Elazig (0.83 ± 0.10µg/g) (Table 1). Also the amount of vitamin E in *Musa sapientum L. Cv Dwarf Cavendish (*English banana) was reported to be 17.53 ± 1.18µg/g. The vitamin E content of *C. laevigata* in this study is thus, higher than that of *Pyracantha coccinea*, cherry and strawberries but lower in *Musa sapientum L. Cv Dwarf Cavendish (*English banana) [31].

Vitamin C is a powerful antioxidant with strong reducing activity. It reacts with superoxide and hydroxyl radical to make them inactive [28]. This study showed the vitamin C content of *C. laevigata* from Malatya, Elazig, and Adiyaman to be, 16.45 ± 1.31µg/g, 35.47 ± 3.54 µg/g and 50.76 ± 2.02µg/g, respectively, in order of increasing concentration (Table 1).

In a similar research vitamin C content was found to range between 110 to 1340 µg/ g in five different species of pulp puree [32]. Based on our findings the vitamin C content of *C. laevigata* is lower than that of strawberry, cherry and pulp puree, however it is higher than that of *Pyracantha coccinea.*

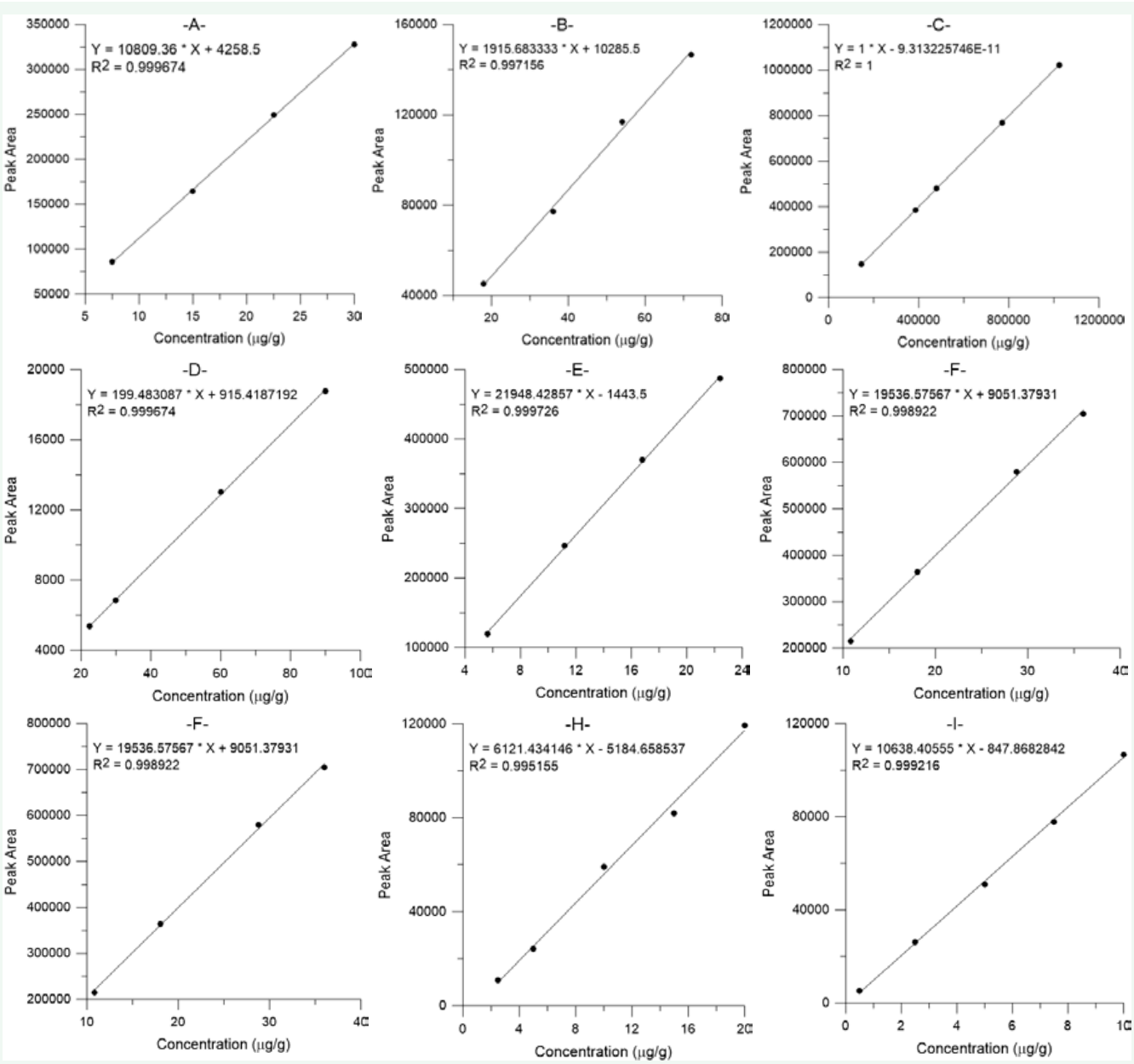

Figure 1 Calibration curves for the Vitamin A (A), Vitamin E (B), Vitamin C (C), Beta Carotene (D), GSH (E), GSSG (F), MDA (G), Lycopene (H), Ghreline (I).

Beta-carotene is a light yellow or orange pigment found in plants and fruits. İt is a precursor of vitamin A. β-carotene turns into vitamin A in intestinal epithelial cells and palmitate. It is stored in the epidermis and in the liver. β-carotene found in carotenoid structure and in terpenoids, is usually found in carrot and green leafy plants[33, 34]. This study showed the Beta-carotene content of *C. laevigata* from Elazig, Adiyaman and Malatya to be 2.88 ± 0.24 µg/g, 3.12 ± 0.28µg/g, 3.87 ± 0.38µg/g, respectively, in order of increasing concentration (Table 1).

In a study of fresh fruit of horseradish, it was reported that β-carotene was found between 12.8 and 37.9 µg/g. It was found that β-carotene was around 37.31 ± 3.61µg/g in the fresh *Pyracantha coccinea* Roemer var. Lalandi [35]. βeta-carotene content was also reported to vary from 5 to 30 µg/g among five varieties of *Mangifera indica L* [32]. Based on this study, β-carotene content of *C. laevigata* is much lower than that of horseradish, *Pyracantha coccinea* and *Mangifera indica L.*

The harmful effects of free radicals are reduced by some substances or completely eliminated. One of these is lycopene. Lycopene, an important carotenoid, is found in fruits and vegetables such as watermelon, pink grapefruit, and in tomatoes (*Lycopersicum esculentum*), giving them red color [36,37]. This study showed the lycopene content of *C. laevigata* in increasing order of concentration to be, 0.44 ± 0.06µg/g, 01.10 ± 0.10µg/g, and2.34 ± 0.18 µg/g,from Adiyaman, Malatya and Elazig, respectively (Table 1). It has been reported that rose hip fruit is rich in lycopene and total lycopene content is between 129 and 352 µg/g in fresh fruit which is significantly higher than that

Table 1: Some parameters in *Crataeguslaevigata* specimens from three different provinces.

Parameters	Elazig	Adiyaman	Malatya
Vitamin A (µg/g)	0.78 ± 0.08	0.76 ± 0.08	1.14 ± 0.11
Vitamin E (µg/g)	0.83 ± 0.10	1.17 ± 0.12	0.89 ± 0.09
Vitamin C (µg/g)	35.47 ± 3.54	16.45 ± 1.31	50.76 ± 2.02
β-carotene (µg/g)	2.88 ± 0.24	3.12 ± 0.28	3.87 ± 0.38
Lycopene (µg/g)	2.34 ± 0.18	0.44 ± 0.06	1.10 ± 0.10
Ghrelin (µg/g)	67.67 ± 6.16	18.96 ± 6.73	79.96 ± 12.14
GSH (µg/g)	82.25 ± 17.26	564.88 ± 81.53	347.52 ± 59.74
GSSG (µg/g)	307.79 ± 52.50	226.60 ± 56.69	118.02 ± 22.39
GSH/GSSG	0.27 ±0.33	2.49 ± 1.44	2.95 ± 2.67
MDA (µg/g)	30.69 ± 6.83	24.27 ± 2.81	26.25 ± 9.95

obtained in *C.laevigata* in this study.In a study of red and dark red fruits, it is reported that lycopene is 8.8 - 42 µg/g in fresh tomato fruit, 23 - 72 µg/g in watermelon fruit, and 33.6 µg/g in grapefruit juice. It was found that lycopene was around 10.67 ± 2.41µg/g in the fresh *Pyracantha coccinea* Roemer var. Lalandi. Based on this study the lycopene content of *C. laevigata* is lower than that of tomato, watermelon, grape fruit and *Pyracantha coccinea* [35,38].

Gherlin, a gastrointestinal polypeptide hormone, acts to stimulate the secretion of growth hormone, gut motility, and gastric acid secretion. İt has been reported to modulate stress, anxiety and improves cardiovascular functions such as vasodilation and cardiac contractility [39]. Tschöp et al. (2000) [40], showed the action of gherlin on the brain where it regulates glucose metabolism, food intake, body weight, and adiposity. The antioxidant effect of gherlin is exhibited in the blood where it increases its total antioxidant capacity [41].

This study shows the Gherlin content of *C. laevigata* from Elazig, Adiyaman and Malatya to be 67.67 ± 6.16 µg/g, 18.96 ± 6.73 µg/g and 79.96 ± 12.14 µg/g, respectively. The gherlin content of fruits from Malatya (79.96 ± 12.14 µg/g) was higher followed by that of Elazig (67.67 ± 6.16 µg/g) then Adiyaman (18.96 ± 6.73 µg/g) (Table 1).

Glutathione is required for cellular activities; Protects cells of the immune system, brain, kidneys, eyes, liver, heart, lungs and skin tissues against oxidative damage. GSH, the aging-delayed and reduced form of glutathione, is the most important antioxidant molecule in the intracellular environment. GSH has many functions such as the de-toxification of xenobiotics, the transport of amino acids, and the reduction of sulfhydryl groups in proteins [42]. In particular, glutathione is the most important metabolite against oxidative stresses in plants and it is found in almost all cells of plants [43].

This study revealed the content of GSH in *C. laevigata*to be 82.25 ± 17.26µg/g, 564.88 ± 81.53µg/g and 347.52 ± 59.74µg/g while the GSSG content was found to be 307.79 ± 52.50 µg/g, 226.60 ± 56.69 µg/g and 118.02 ± 22.39 µg/g from Elazig, Adiyaman and Malatya respectively. However, the GSH/GSSG ratio was found to be 0.27 ± 0.33µg/g, 2.49 ± 1.44 µg/g, and 2.95 ± 2.67 µg/g. The GSH content of *C. laevigata* from Adiyaman (564.88 ± 81.53 µg/g) had the highest concentration followed by Malatya (347.52 ± 59.74 µg/g) then that of Elazig (82.25 ± 17.26 µg/g). On the other hand, the GSSG content of Elazig (307.79 ± 52.50 µg/g) is higher followed by that of Adiyaman (226.60 ± 56.69 µg/g), then Malatya (118.02 ± 22.39µg/g) (Table 1). The level of glutathione in *Musa paradisiaca L.* (plantain) was reported to be 54.10 ± 0.60µg/g [31].

The amount of GSH in the strawberry fruit was 722.25 ± 23.19 µg/g while the amount of GSSG was determined as 17.13 ± 2.59 µg/g [44]. GSH was found to be 200.81 ± 30.15 µg/g and GSSG was 47.53 ± 5.45 µg/g in the fresh *Pyracantha coccinea* Roemer var. Lalandi [35]. Based on this study the GSH content is lower than that of strawberry while the GSSG content of *C. laevigata* is higher. The concentration of GSSG and GSH/GSSG ratio are valuable indicators of oxidative stress in cells and tissues because exposing cells to increased levels of oxidative stress causes the buildup of GSSG and the ratio of GSH to GSSG will decrease [45]. Free radicals affect lipid peroxidation by acting on unsaturated fatty acids in the membranes. The resulting lipid peroxides break down rapidly to form reactive carbon compounds [46]. The most important of these reactive carbon compounds is MDA [47]. In this study, the MDA content of *C. laevatiga* from Elazig, Adiyaman and Malatya was found to be 30.69 ± 6.83µg/g, 24.27 ± 2.81µg/g and 26.25 ± 9.95 µg/g respectively. The MDA content of *C. laevigata* from Elazig (30.69 ± 6.83µg/g) was higher followed by that of Malatya (26.25 ± 9.95 µg/g) then Adiyaman (24.27 ± 2.81µg/g). It was found that MDA was around 7.70 ± 0.89 µg/g µg/g in the fresh *Pyracantha coccinea* Roemer var. Lalandi. The MDA content of *C. laevigata* from all three locations are higher than that of *Pyracantha coccinea* Roemer var. Lalandi [35].

CONCLUSION

The observed differences in the values obtained from one province to another may be attributed to differences in enviromental factors like geographical location, soil, temperature and altitude. These factors play an important role in the metabolism and concentration of bioactive compounds in plants as they control the types and quantity of such compounds in line with these enviromenta lvariations [48]. The findings observed in this study on the amount of these compounds (vitamins A, E, C, β-carotene; GSH, GSSG, GSH/GSSG; Lycopene, Gherlin and MDA) in *Craetagus laevigata* fruit reiterates its nutritional and economical value. It can act as a natural, cheap and accessible source of important antioxidants which can protect the body

against oxidative stress that lead to aging and degenerative diseases such as cancer and diabetes. It has a future potential in the functional food industry as it can be processed into various value added products where its antioxdant value can be harnessed and optimised to suit a wide variety of consumer preferences.

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