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Effects of height and organs on flavonoids of *Crataegus microphylla* C. Koch in Iran

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Abstract

This study was conducted to evaluate the effect of height and organs on the amount of two flavonoids in Rutin and Quercetin in three height lines of 100, 1000 and 2000 m in the heights of the north of Iran. To study the effect of height and organ on the amount of two flavonoids of Rutin and Quercetin, the simple nested experimental design was used and in each height two organs (flower and leaf) was studied to measure Rutin and Quercetin and all the plant organs were used to measure the total flavonoids. The average comparisons determined that 100 m height has the highest amount of Rutin and Quercetin also a significant difference was observed in 1% level between the amount of Rutin and Quercetin in flowers and leaves at different heights. The amount of Rutin in all three heights was in the leaf more than in the flower, but for Quercetin the reverse was true so that the amount of this material in all three heights were in the flower more than in the leaf. The effect of height on the amount of total flavonoids was significant in 1% level as its highest amount was observed at 1000 m height.

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Introduction

Crataegus microphylla C. Koch commonly called hawthorn or thornapple, is a large genus of shrubs and trees belonging to, Rosaceae, native to temperate regions of the Northern Hemisphere in Europe, Asia and North America. They are mostly growing to 5–15 metres tall with small pome fruit and (usually) thorny branches, Phipps *et al*., (2003). In Iran *Crataegus microphylla* C. Koch generally growth in Hyrcanian forests in north of Iran and use in traditional and naturopathic medicine as a digestive aid, promotes blood circulation, and reduce blood stasis, Amin (2005). Recent research has shown that polyphenols derived from the fruit of genus *Crataegus* have anti-tumor activities on skin, indicating a potential use in preventing skin cancer, Kao *et al*., (2007). The medicinal parts of this plant are leaves, flowers and fruits and the main compounds are flavinoids, triterpene, saponins, organic acids, and amines, Barceloux (2012). Many of medicine characteristics of *Crataegus microphylla* refers to flavonoids. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants, Spencer *et al.,* (2008). Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, anti-inflammatory, Yamamoto *et al*., (2001) anti-microbial, Cushnie *et al* .,(2005, 2011) anti-cancer, De Sousa *et al. ,* (2007) and anti-diarrheal activities, Schuier *et al*., (2005). Since the ecological condition could be effects on quality and quantity of plants chemical composition, Hemmati *et al*.,(2005) in this research we determine the total flavonoids and amount of Rutin and Quercetin flavonols in different organs (leaf and flower) of *Crataegus microphylla* in north of Iran in three different heights.

Materials and methods

This study was conducted to evaluate the effect of height and organs on the amount of two flavonoids in Rutin and Quercetin in three height lines of 100, 1000 and 2000 m in the heights of the central Alborz in Kojur with geographic coordinates of 51°, 25′, 00″ to 51°, 25′, 30″ longitude and 36°, 16′, 00″ to 36°, 18′, 30″ latitude. To study the plant flavonoids, the samples of leaf and flower were taken from *Crataegus microphylla*, in three height lines of 100, 1000 and 2000 m in spring. In order to obtain the whole extract, the amount of 1 gram of powdered sample was located in 50 ° C water bath with 95% methanol for 20 minutes and the volume of the solution obtained was measured after passing through filter paper. 10 ml extract was obtained from each sample and poured into the small and matte lidded glass and kept in the refrigerator until the injection into high performance liquid chromatography device. To measure the flavonoids in the whole extract, the high performance liquid chromatography device (HPLC) with the following characteristics was used: Pump: Knauer K-1001, Degasser: Knauer K-1001, Solvent Organizer: Knauer K-1500, Column: C-18 25*0.46 cm Teknokroma, Detector: Knauer UV K-2501. To study the effect of plant height and organ on the amount of two flavonoids of Rutin and Quercetin, the simple nested experimental design was used and in each height (100, 1000 and 2000 m) two organs (flower and leaf) was studied to measure Rutin and Quercetin. All the plant organs were used to measure the total flavonoids. The normality test of error distribution, analysis of variance and the average comparison (LSD) of data was conducted by using SAS 9.0 software. Also the characteristics of climate including rainfall, temperature and wind speed were measured.

Results and discussion

According to De Martonne method, the climate of region is semi-humid. The amount of rainfall in the area studied is over 500 mm per year and the highest rainfall occurs in the spring and in April that the

rainfall in this month of the year reaches to more than 80 mm. The lowest rainfall occurs in the summer and in September. In this region, the number of rainy days is more than 100 days, and the number of frost days is less than 100 days. In terms of temperature, the coldest month is January that the minimum temperature in this month is less than - 10 ° C and the maximum temperature in this month is more than 15°C. The average daily temperature in the coldest months of the year reaches to 3.7 ° C. The warmest month of year is August in which the minimum temperature is 11°C and maximum temperature is more than 32°C. The average daily temperature in the warmest month of the year reaches to 20°C. The average wind speed is more than 15 m/h during the year and its direction is mainly northwest. All characteristics studied in the 1% probability level followed the normal distribution. Analysis of variance showed that there is a significant difference between different heights regarding the amount of Rutin and Quercetin in 5% and 1% probability levels, respectively (Table 1).

Table1. Variance analysis of the effect of height and organs on Rutin and Quercetin.

Source of variation	Df	Mean of Square	
		Rutin	Quercetin
height	2	0.0029 *	0.1002 **
Organs* height	3	0.00379 **	0.0322 **
Error	12	0.00057	0.00043
CV%	-	7.87	7.64

* Significant difference in 5% probability level

** Significant difference in 1% probability level

The average comparisons determined that 100 m height has the highest amount of Rutin and Quercetin (Table 2). Also a significant difference was observed in 1% level between the amount of Rutin and Quercetin in flowers and leaves at different heights.

So the amount of Rutin in all three heights was in the leaf more than in the flower, but for Quercetin the reverse was true so that the amount of this material in all three heights were in the flower more than in the leaf. The effect of height on the amount of total flavonoids was significant in 1% level (Table 3) as its highest amount was observed at 1000 m height (Table 4).

Table 2. Average comparisons of the characteristics in different height.

Characteristics Height	Rutin	Quercetin
100	0.103 a	0.419 a
1000	0.093 b	0.217 b
2000	0.090 c	0.179 b

Same letters show non-significant difference

Different letters show significant difference

Table 3. Variance analysis of the effect of height and organs on total of flavonoids.

Source of variation	df	Mean of Square(Total Flavonoids)
Height	2	0.177 **
Error	6	0.00003
CV%		1.17

** Significant difference in 1% probability level

Table 4. Average comparisons of flavonoids in different heights.

characteristics Height	Total Flavonoids
100	0.455 c
1000	0.603 a
2000	0.494 b

Different letters show significant differences.

Flovonoids production in different organs in plants may change by enzymes activity, Peter *et al*., (1993) and hormones such as cytokinin and gibberellic acid could be active a few flavonoids production enzymes, Seymour *et al*. (1993) as Rio *et al.,* (1995) with used of cytokinin increased the amount of flovonoids in unripe fruits. Other research show temperature could be effect on flovonoids so that some flovonoids of citrus in cold areas is more than tropical areas Davise *et al.,* (1994). So changing the amount of flavonoids of Rutin and Quercetin in different organs in this research is similar to mentioned researches. Considering that for every 100 m increase in height the air temperature decreased 0.5 °C (Met. Office 2007) and also considering the changes in other ecological factors, it seems that changing the ecological factors arising of height lead to changing metabolic and enzymatic activities of plant s and effects to the amount of flavonoids of Rutin and Quercetin at different heights and organs in *Crataegus microphylla* that confirm the results of Hemmati *et al.,* (2005).

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