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Evaluation of the Morphological and Biochemical Properties of the Different Plant Organs of Nettle in Two Provinces of Mazandaran and Golestan

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Abstract

In this study the effect of altitude (in two provinces of Mazandaran and Golestan) on some morphological and biochemical properties of the stems, flowers and root of nettle plant was examined. Sampling was performed at the flowering time of plant. Extraction and identification of active ingredients were performed by using spectrophotometer and HPLC methods. This research was conducted based on completely randomized design by using statistical analysis software (SAS, 9.1). Results that altitude did not show significant effect on morphological properties, but in general it can be said that the height 1450m above the sea level in both provinces was the best height for morphological characteristics. Maximum size of stem diameter (7.05cm), root diameter (5.01cm), root length (31.1cm) and number of inflorescence (54.2) were observed in Golestan province at the altitude of 1450m and the highest size of inflorescence (5.52cm) in the Mazandaran province was related to the altitude of 1450m above the sea levels. The amount of total phenol, total flavonoids, Chlorogenic acid, caffeic acid and rutin in the stem and leaf, nettle root increased with increasing the height, so that the largest amount of total phenol (3.38 and 1.28 mg/g), total flavonoids (3.98 and 2.53 mg/g), Chlorogenic Acid (0.3 and 0.13 mg/g), caffeic acid (0.065 and 0.021 mg/g) and rutin (0.37 mg/g) were observed in stem, flower and root plant at altitude of 2250 m in leh koh region of Mazandaran province. Results showed that positive correlations exist between total phenol and flavonoid content and height of sea level. Many of morphological parameters such as stem diameter, root diameter, root length and number of inflorescence in Golestan province increased with increasing altitude up to 1450 m and the size of inflorescence in Mazandaran province in the same height showed the highest value.

1. Introduction

Urtica dioica L. that commonly known as nettle or stinging nettle is a plant frequently used by humans for medicinal purposes. This herb belongs to the family of Urticaceae and occurs as a perennial plant in temperate zones of Asia, America, and Europe (Asgarpanah & Mohajerani., 2012). Nettle Reproduced through implant seed in spring or summer, and planting pieces rooted in a fall occurs (Zargary., 2004). Public nettle plant was observed in the wet spots region of Iran especially in northern, western and central areas such as Isfahan, Shahrood, Bastam and Kashan. Plant medicinal sections comprise leaves, roots, seeds and latex (Zargary., 2004). Several phenolic compounds are available in the nettle such as caffeic acid, fluric acid, sinapeak acid, fisettin, Myrcetine (Mahmoudi *et al*., 2007). The most effective environmental factors on quantity and quality of ingredient plants are light, temperature, irrigation and height in the place (Omid beigi., 2005). Hemmati *et al*., (2007) studied the effects of climate on flavonoids content in different organs of hawthorn shrubs. They showed that, there is an interaction between location and characters and they concluded that the amount of Quercetin in hawthorn plant in Kalardasht-Mazandaran was higher than Gorgan. In another study, Jaimand *et al*., (2009) studied the amount of flavonoid compounds, Kaempferol and Quercetin in ten genotypes of damask rose, in western region of Iran. They found that the best region in terms

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of Kaempferol and Quercetin compounds were west Azerbaijan, Ilam and Ardabil. Karimi *et al*., (2010) studied the amount of compounds of thyme in Isfahan and Chaharmahal-bakhtiari provinces and expressed that the highest level of Thymol was related to sheikh shaban with 3747 m height from the sea level and the highest amount of carvacrol related to the sample located at the altitude of 2370 m from the sea level. They also stated that the altitude has significant effect on Thymol amount while has not significant effect on carvacrol amount. Tajale *et al*., (2002) investigated the effects of height on phenolic and flavonoid compounds in different organs of *Carateagus microphylla* plant and stated that this plant in the altitude 1000m had higher levels of phenol and flavonoid content than plants grown at lower altitude. chasemi *et al*., (2011) studied the effects of environmental factors on the antioxidant activity and the amount of total phenol and flavonoids content in walnuts plant (*Juglans Regia*). They stated that the highest amount of total phenol and flavonoid compounds was related to Abali region with the highest altitude and lowest average of daily temperature. Although primary and secondary metabolites are influenced by genetic factors but production of these materials are influenced by environmental factors too. They showed that environmental factors are the most important factors affecting the level of expression of biosynthesis genes responsible for production of secondary compounds in herbs (Sahar khiz., 2002). The aim of this research is to determine the best altitude and medicinal organs nettle in terms of total phenol and flavonoid compounds.

2. Material and Methods

2.1. Collection and Identification of Plants

In this research, wild plants were gathered in different altitudes of Mazandaran and Golestan province. After selection of regions, three populations were collected randomly from each region in Mazandaran and Golestan province. Samples transferred to horticulture laboratory of Gorgan University of Agricultural Sciences and Natural Resources, Iran, and identified based on the keys botany and flora Iran and resources (Ghahreman., 1993) by botanical specialists.

2.2. Characteristics of the Studied Areas

Geographical location and height of the sample collection location in each area was determined using an GPS device, and characterization of climatic condition based on the nearest weather station to habitat and the statistics long-term meteorological parameter were prepared (Table 1). To determine the most important characteristics of soil in each habitat, soil samples randomly from depth zero to 30 cm were collected and transferred to soil science Laboratory for soil test. The most important soil science properties such as electrical conductivity (EC), Soil texture, organic matter and pH were measured in collected soils. (Table 1). Before harvest time, several parameters including plant height, root Length, Stem diameter, root diameter, number of inflorescences per plant and Inflorescence size of Nettle plants were measured.

Table1 Climatic characteristics in the study sites Nettle

Physical and Chemical properties of Soil					Latitude	Longitude	Altitude sea level (m)	Area	Province
Land use	Organic matter(%)	Soil texture	pH	EC (ds.m)					
Agricultural	3.22	Clay	7.91	1.48	E54˚42´	N36˚33´	5	Babol- Haydarkola	Mazandran
Garden	3.96	Loam	7.73	1.28	E52˚39´	N36˚11´	750	Babol- Firozjay	Mazandran
Grassland	3.37	Loam Silty	7.14	1.87	E52˚36´	N36˚8´	1450	Babol- Shikhmosa	Mazandran
Grassland	4.04	Loam	7.68	1.42	E52˚34´	N36˚6´	2450	Babol- Lehkoh	Mazandran
Agricultural	2.25	Clay Loam	7.48	1.36	E54˚23´	N36˚5´	50	Gorgan- Afsaran	Golestan
Silvan	2.21	Loam Sandy	7.34	1.07	E54˚29´	N36˚43´	750	Gorgan- Naharkhoran	Golestan
Grassland	3.83	Loam Clay Silty	7.58	1.47	E54˚27´	N36˚41´	1450	Gorgan- Zyarat	Golestan
grassland	0.874	Loam Sandy	7.95	2.32	E54˚31´	N36˚38´	2250	Gorgan- Chaharbagh	Golestan

Biochemical properties including Phenol and flavonoid compounds were determined using spectrophotometry method. Chlorogenic and cafeic acids and rutin were determined with HPLC method.

2.3. Extraction Procedure

The leaf samples were collected from the experimental plants and dried at room temperature. Dried samples were powdered and each sample [0.5 g] was extracted by percolation method using pure methanol [5 mL] for 24h to have a complete solvent removal extract.

2.4. Total phenolic and Flavonoid Contents

Total phenolic compounds were assayed using the Folin–Ciocalteau reagent (Trajtemberg *et al*.,2006). The extract of sample was added to 0.5 ml of distilled water and was mixed with 5 ml of the Folin–Ciocalteau reagent and aqueous Na_2CO_3 [4 mL, 1M]. The mixture was allowed to stand for 15 min and the phenols were determined spectrophotometrically at 760 nm. Total phenolic content of plant parts was expressed as mg of gallic acid equivalents per gram of dry weight [mg GAE g−1 DW] through the calibration curve with gallic acid. All samples were analyzed in three replications. Colorimetric aluminum chloride method was used for flavonoid compound determination (Ghahreman, 1979-2000). Briefly, 0.5 mL of each plant extracts in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2 mL of distilled water and left at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with Camspec M501 double Beam Scanning UV/Vis Spectrophotometer. Total flavonoid content was calculated as quercetin via a calibration curve. The calibration curve was prepared by preparing quercetin solutions at concentration of 12.5 to 100 mg mL^{-1} in methanol.

2.5. Analysis of Chlorogenic, Caffeic Acid and Rutin by HPLC Method

The Chlorogenic and Caffeic acid content as two the important substance of Cynarin and Cynaroside were determinate using HPLC method. Standards were dissolved in methanol. The extract solutions were filtered through 0.45 µM filter [Whatman type]. The HPLC analysis was carried out on a Merck Hitachi apparatus model Lachrom L-7100 connected to a computer analytical program HSM and a RP C18 column [250 x 4.6mm, 5 µM] was set at 40 ^{0}C. Mobile phase included filtered H_3PO_4 [Phosphoric acid 0.5%], acetonitril and deionized water. The flow rate was kept at 1 mL per min. UV detector at 280 nm was used for Chlorogenic acid: [9 min] and caffeic acid [14 min]. The mobile phase used for rutin measurement was consist of 50 ml pure methanol merck, 49 ml of deionize distilled water and 1 ml of acetic acid with a flow rate in 1ml/min. By comparing the retention time and area under the curve of the sample with standard sample, the amount Caffeic acid, Chlorogenic acid and rutin were determined and expressed based on the ml/g of dry weight (Trajytnbraget *et al*.,2006; Santoz-gamz *et el.*, 2003).

3.1. Statistical Analysis

The effect of height parameter on morphological and biochemical characteristics of the nettle plant has been investigated using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5% significant level using the SAS software (2001) program. For the morphological characteristics five replications and for biochemical properties three replications were used.

4. Results

Results showed that there was no significant difference between two provinces of Mazandaran and Golestan in all traits except in number of inflorescence, but the effect of altitude in each county, in all traits were significant (Table 2).

The highest stem diameter (7.05 and 6.98mm) were found at the altitude of 1450 and 2250 m in Golestan province and the lowest amount of stem diameter (4.44mm) was related to the altitude of 10 m in Mazandaran province.

Table 2 Statistical analysis of morphological characteristics

Source of variation	DF	Inflorescence size	Number of inflorescences in plant	Stem diameter	Root diameter	Root length	Plant height
Province	1	0.55^{ns}	295^{*}	0.41^{ns}	0.06^{ns}	20.37^{ns}	8.55^{ns}
Altitude in Province	6	1.34^{*}	262^{*}	3.08^{**}	1.91^{**}	102^{**}	1226^{**}
Error	32	0.57	99.62	0.73	0.23	16.87	372.6
CV %		18.5	26.12	14.77	11.6	17.63	18.5

^{ns}, * and ** are not significant, significant at 5 and 1%, respectively.

The maximum and minimum amount of root diameter was observed at the altitude of 1450 m in Golestan and 750 m in Golestan province, respectively. The minimum and maximum amount of root length was observed at the altitude of 2250 and 1450 m in Golestan province respectively (Table 3). Between the two provinces in terms of inflorescence number, significant difference ($p<0.05$) was observed (table 2). The average number of inflorescences was 46.8 numbers in Golestan pronince, while in the Mazandaran was 41.35 numbers. Interaction of altitude and province, showed that Golestan province with altitude 1450 m had the largest number of inflorescence (54.2 numbers) while at the altitude of 750 m in Mazandaran province the lowest number of inflorescence (35.8 numbers) was observed (Table 3). Positive correlations were observed between numbers of inflorescence in the samples with stem diameter and root length. Inflorescence size was almost identical in samples, only in the height of 1450 m in Mazandaran, inflorescence size was higher (5.52cm) than other samples. Results showed that increase in altitude above the sea level can be reduced the height of the plan. The highest plant height was observed in the Mazandaran province with the height of 750 m while the lowest amount of this parameter (77.2) was related to altitude 2250 m in Mazandaran (Table 3).

4.1. Biochemical Parameters

Variance analysis results of biochemical characteristics revealed that the interaction effects between altitudes and province had significant on all biochemical parameters (Table 4).

4.2. Total Phenolic Compounds in Stems, Flowers and Roots

The amount of total phenol in stems and flowers were affected by the height so that the maximum phenolic compounds (3.38mg/g) was observed at the altitude of 2250 m in Mazandaran and the lowest amount of phenolic compounds (1.48 mg/g) was related to the height of 50 m in the Golestan province (Fig. 1). The amount of total phenol in roots than other parts showed a marked decline and even fewer differences were observed at different heights, although the greatest amount of total phenol in root (1.21 mg/g) was observed at the altitude of 2250 m in Mazandaran and the lowest amount (1.09 mg/g) related to the altitude of 750 m in Golestan province (Fig. 2).

Table 3 Analysis of morphological characteristics in Mazandran and Golestan provinces

Inflorescence size (cm)	Number of inflorescences	Plant height (cm)	Root length (cm)	Root diameter (mm)	Stem diameter (mm)	Height * Province
4.15b	42.4b	118.8a	26.24a	4.5a	4.44c	
4.21b	35.8c	120.3a	20.6b	4.33ab	5.77b	Mazandran- 750m
5.52a	48.2ab	102.9c	23.3ab	4.07ab	5.6b	Mazandran-1450m
4.6b	39c	77.2d	20.2[b]	3.9b	5.11b	Mazandran-2250m
4.14b	46.6b	116.4ab	25.6ab	4.4ab	5.7b	Golestan- 50m
4.11b	38.4c	103c	21.75b	3.02bc	5.38b	Golestan- 750m
4.91b	54.2a	93.3cd	31.1a	5.01a	7.05a	Golestan- 1450m
4.38b	48ab	88.8d	17.6bc	4.06ab	6.98a	Golestan- 2250m

In each column means with same letter are not significantly different ($p> 0.05$).

Table 4 Statistical analysis of biochemical characteristics

Source of variation	DF	Phenolic compounds	Root Phenol	Flavonoeid compounds	Root Flavonoeid
Province	1	0.003^{ns}	0.002^{ns}	0.001^{ns}	0.001^{ns}
Altitude in Province	6	1.21^{**}	0.008^{**}	0.34^{**}	0.87^{**}
Error	16	0.007	0.002	0.003	0.002
CV %		4.17	1.38	1.93	2.1

^{ns}, * and ** are not significant, significant at 5 and 1%, respectively.

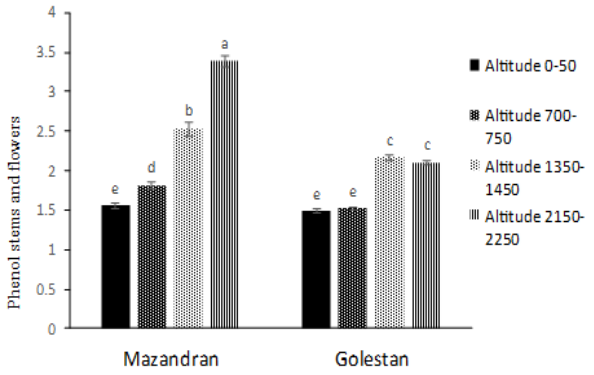

Fig. 1 Comparison of interaction effects between Climate and province on stem and flower phenol in Nettle plant (mg/g)
Means with a same letter in are not significantly different (p> 0.05).

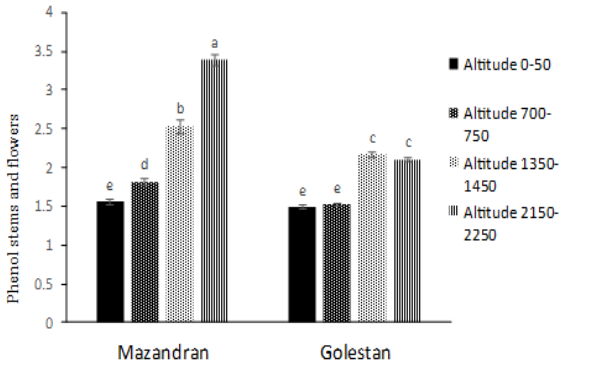

Fig. 2 Comparison of interaction effects between Climate and province on total phenol of root in Nettle plant(mg/g)
Means with a same letter in are not significantly different (p> 0.05).

The highest amount of total flavonoid content observed in stem and flower (3.98 mg/g) at the altitude of 2250 m in Mazandaran and by reducing the height to 1450 m its amount was reduced but in samples from other area the amount of this compound almost were identical and has not significant difference together (Fig 3).

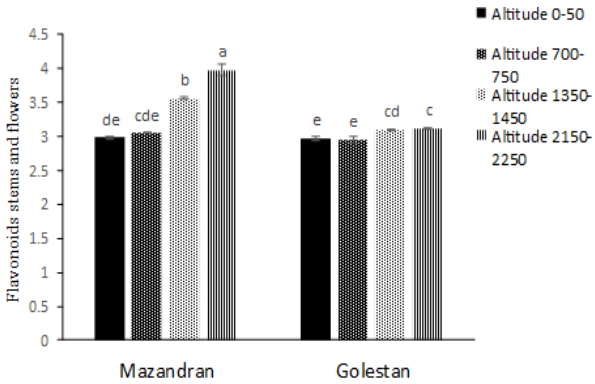

Fig. 3 Comparison of interaction effects between Climate and province on stem and flower flavonoid content in Nettle plant(mg/g)
Means with a same letter in are not significantly different (p> 0.05).

Total flavonoid content in root was lower than stem and flower so that the maximum amount of flavonoid compounds (2.53 mg/g) was obtained at the altitude of 2250 m in Mazandaran and other areas of two provinces have the same amounts of total flavonoid in root. The lowest amount of this material (2.1 mg/g) observed at the height of 10 m (Fig. 4).

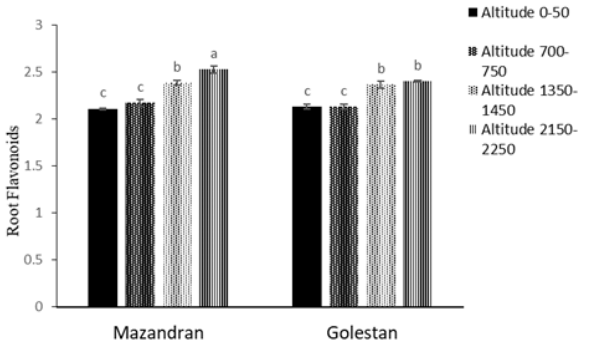

Fig. 4 Comparison of interaction effects between Climate and province on root flavonoid in Nettle plant
Means with a same letter in are not significantly different (p> 0.05).

4.3. Chlorogenic Acid Content of Roots, Stems, and Flowers

In the stem and flower organs, the highest amount of chlorogenic acid was observed at high altitude, so that the highest amount of this material (0.23mg/g) related to the sites 2250 m in Mazandaran, the lowest amount of chlorogenic acid in stems and flowers (0.017mg/g) were observed at height of 10 m in golestan (Fig 5).

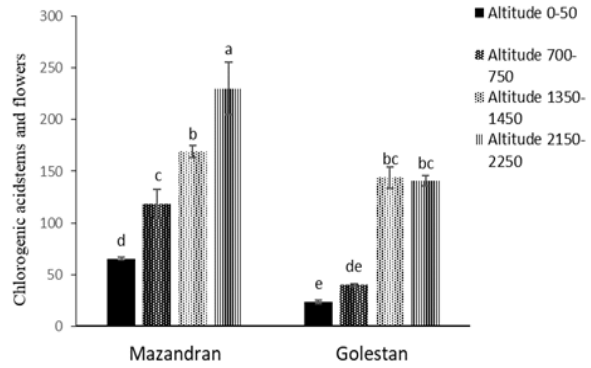

Fig. 5 Comparison of interaction effects between Climate and province on stem and flower chlorogenic acid in Nettle plant (µg/g)
Means with a same letter in are not significantly different (p> 0.05).

The amount of chlorogenic acid in Root nettle was lower than the aerial organs, so that maximum amount of this material (0.0107mg/g) was observed at altitude of 2250 m in Mazandaran and by reducing the height, the amount of this material in roots decreased considerably so that at the altitude of 50 m in Golestan province its content reached to 0.0038 mg/g (Fig. 6).

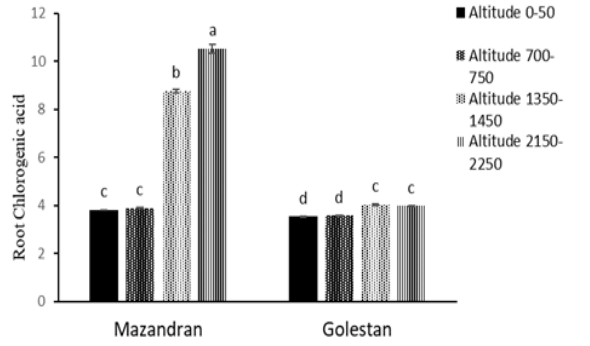

Fig. 6 Comparison of interaction effects between Climate and province on root chlorogenic acid in Nettle plant(µg/g)
Means with a same letter in are not significantly different (p> 0.05).

4.4. Caffeic Acid Content in Roots, Stems and Flowers

The amount of caffeic acid in the stem and flower organs also was affected by altitude level so that the highest amount of caffeic acid (0.0065 mg/g) observed in the altitude of 2250 m in Mazandaran province. Other lands in two provinces were placed in the second position in this case and the lowest amount of caffic acid (0.0046mg/g) was related to the lowest altitude in Golestan and Mazandaran respectively (Fig. 7).

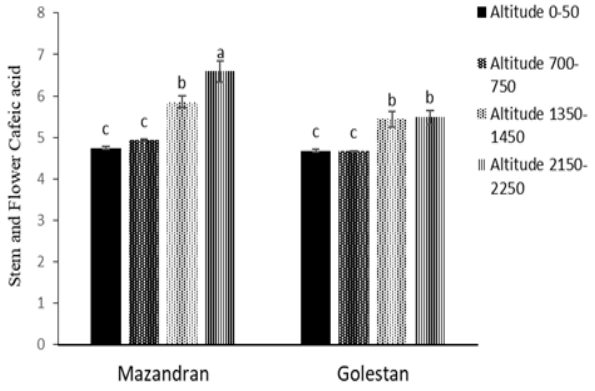

Fig. 7 Comparison of interaction effects between Climate and province on stem and flower cafeic acid in Nettle plant (µg/g)
Means with a same letter in are not significantly different (p> 0.05).

The result showed that the amount of caffeic acid in nettle root was lower than other organs and its amount was very small at lower altitude. The maximum amount of caffeic acid (0.0041 mg/g) was observed at height of 2250 m and 1450 m in Mazandaran and high lands of Golestan province in the next category groups (Fig. 8).

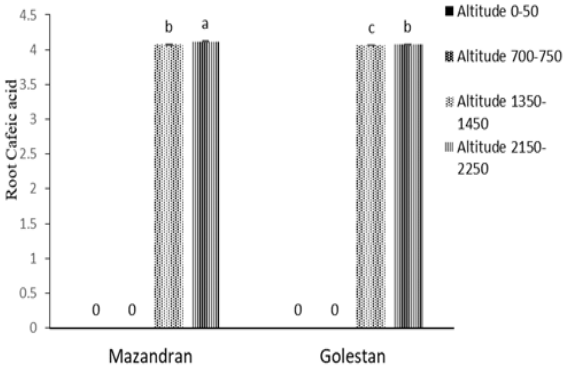

Fig. 8 Comparison of interaction effects between Climate and province on root cafeic acid in Nettle plant (µg/g) Means with a same letter in are not significantly different (p> 0.05).

4.5. Rutin Content in Roots, Stems and Flowers

Result of mean comparison showed that the effects of altitude and province on the level of rutin content in stems and flowers were significant (p<0.05). The highest amount of rutin (0.0067 mg/g) was related to the altitude of 2250 m in Mazandaran and the lowest amount (0.0017mg/g) observed at the altitude of 750 m in Golestan (Fig. 9). According to the results, no rutin was observed in the nettle root.

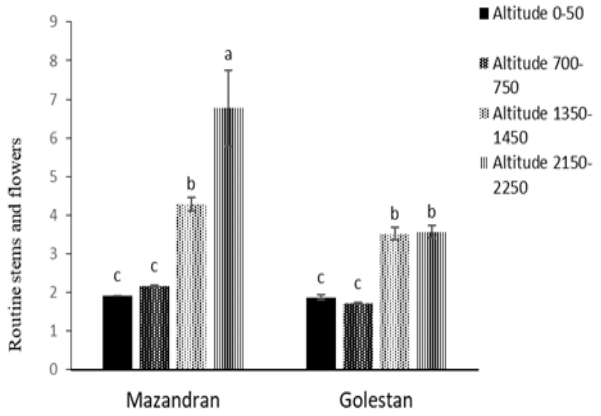

Fig. 9 Comparison of interaction effects between Climate and province on stem and flower routine in Nettle plant (µg/g)
Means with a same letter in are not significantly different (p> 0.05).

4.6. The Relationship Regression of Height of Sea Level with Amount of Total Phenol and Flavonoid Content

In the stem and flower organs there is significant positive correlation between the amount of total phenol

with height above sea level (R^2=0.95), so that in the altitudes higher than 1000 m, the amount of total phenol content with more regression slope than total flavonoid content (Fig. 10). In the nettle root plant there is lower amount of total phenol than total flavonoid (r=0/87) (Fig. 11).

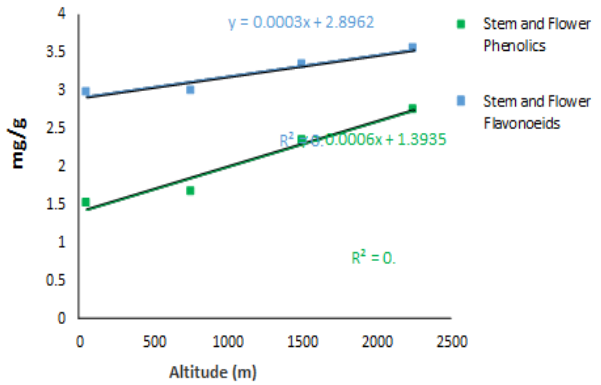

Fig. 10 The relationship between altitude with total phenol and flavonoids compounds in the nettle plant stem and flowers

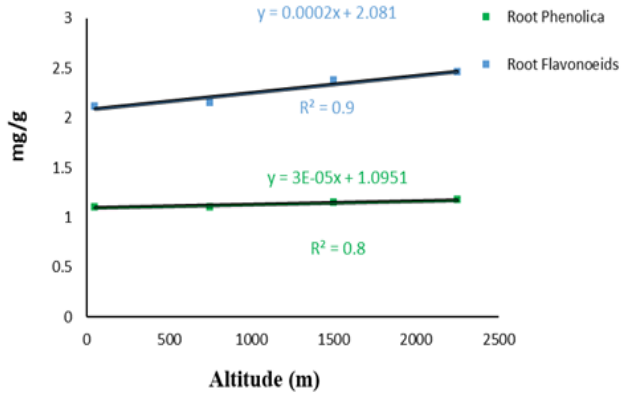

Fig. 11 The relationship between altitude with total phenol and flavonoids compounds in the nettle plant root

5. Discussion

In the present study the quantity and quality of active ingredient in different nettle organs in two provinces of Iran at the different height were studied. Results achieved of this study showed a significant effect of interaction between height and province. The amount of total phenol and flavonoids content and as well as its dependent with the altitude have significant positive correlation, so that the highest amount of these combinations was observed in the leh koh Babol area. Temperature and relative humidity decreased with increasing altitude. A decrease in temperature and humidity can be factors in the increase of phenol and flavonoid compounds. However, in the mountain leh koh organic matter was higher compared to other regions. Although in leh koh area the amount of organic material was higher than other regions (Table 1). The researchers said that in areas with cooler weather than in warm regions is higher in flavonoids because the cooler temperature increases during cell division (Davis & albergo., 1994). The various studies on the effect of habitat on the amount of secondary metabolites in different plant showed that in most case the role of habitat as a factor influencing secondary metabolite accumulation is emphasized.location of plant growth can be effective through changes in temperature and humidity to the process of forming of the ingredients. The mechanism of environmental factors effect on the accumulation of secondary metabolites is not clear, however the impact of environment in the process of metabolic production is clear and also it has factors related to the production of enzymes and chemical reaction (Hemmati *et al*., 2003; Syvstava & Shim., 2002). Ghasemi *et al* (2011) stated that in walnut plant with increasing of height level the amount of phenolic substances in the plant increased. According to the figures1-4 the amount of phenolic and flavonid compounds in different organs of a plant are not a ratio and amount of these materials in the stem and flower organs is higher than roots. The amount of ingredients in the plant organs is not constant and related to the stages of plant growth and in some circumstances can be changed. The quantity and quality of chemical compounds is dependent on plant variety, environmental conditions variation and plant phenology (Plati *et al*., 2005). Also there is a large difference in the amount of plant ingredients in same species growing in different conditions (Hanlido *et al*., 1992). Hemmati *et al* (2007) stated that the various organs are different together in term of flavonoid compound production. They stated that the most amount qristin is related to the flower organ. Azar yavand *et al* (2009) studied the essential oil composition of leaves and flower in Achillea herb in the different heights of the Seyah beshe redion in Mazandaran province. They concluded that the height level effect the amount of essential oil in this plant so that the amount of essential oil compounds in the higher altitude is more and in the flower organ is higher than leaf. Avmah and Maza (1996) showed that with increasing of height the amount of the flavonoid compound in herb organs increased. Production of phenol and flavonoid compounds in the various organs may be due to change in some of the enzyme activities and its role in precursor (Peter & Richard., 1993). Increasing the Flavonoid compound in flower and stem organs than the root, can

be due to the change in enzyme activity and its higher role in precursor which in aerial organs than ground organs. In agreement with this hypothesis other researchers noted that growth regulators such as cytokine and Gibberellic acid hormones may be the enzyme producer of flavonoid compound (Seymour *et al*., 1993). In same respect Del Rio *et al* (1995) of synthetic cytokine (benzyl amino purine) at different stages of fruit growth use and concluded that enzyme activities such as hydrolase, methyl transfers and ramnuzyl transferase increased directly or indirectly and lead to increase accumulation of flavonoid compounds in fruit tissues premature. The most important phenolic compound in thyme is thymol (Joshed *et al*., 2006; Stahl Biskup., 1995). Karimi *et al* (2010) demonstrated that thymol and height above sea level there is a positive relationship.

In this study the effect of altitude, on morphological and biochemical characteristics of nettle plant was determined and found that there was no significant difference between Mazandaran and Golestan provinces but the interaction of height in each province (Mazandaran and Golestan) on the studied treatment were significant. Many of morphological parameters such as stem diameter, root diameter, root length and number of inflorescence in Golestan province increased with increasing altitude up to1450 m and the size of inflorescence in Mazandaran province in the same height showed the highest value. It can be said that the height of 1450 m above the sea level was the best height for morphological properties. In term of the amount of phenolic and flavonoid compounds, at high level, chlorogenic acid, caffeic acid and rutin increased significantly. The highest amount of total phenol, total flavonoids, chlorogenic acid, caffeic acid and rutin in the stems, flowers and root of the plant were observed at height of 2250 m at the area leh koh in the Mazandaran province. T correlation between amount of phenolic and flavonoid compounds in stem and flowers was a significant positive correlation but this correlation was observed below in the root.

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