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# Evaluation content of flavonoids and anthocyanins in Iranian borage (*Echium amoenum* Fich & Mey) subjected in eshkevari accessions affected by different habitats in North of Iran

**Esmail Babakhanzadeh Sajirani[1*], Javad Hadian[2], VahidAbdossi[1], Kambiz Larijani[3]**

[1]*Department of Horticulture Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agriculture, Medicinal Plants and Drug Research Institute, ShahidBeheshti University, G. C., Evin, Tehran, 1483963113, Iran*

[3]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*

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**Abstract**

Iranian GoleGavZaban (*Echium amoenum* fich&mey) is a perennial endemic Iranian medicinal plant belongs to Boraginaceae family and naturally grown in northern mountainous of iran. The violet dry petals have been used as medicinal purposes in folk Iranian medicine. This study is the first report about the analysis amount of flavonoids and anthocyanins of *Echium amoenum* in eshkevari accessions. Considering that variety of different habitats and climates have different effects on growth and plant ingredients, in this study, plant samples at 10 accessions with different altitude were collected from eshkevari in North of Iran.The results of regression analysis and co-efficient of determination showed that the anthocyanin content (R2 = 0.847) and flavonoids (R2 = 0.873) in different accessions have been severely affected by altitude. With increasing altitude, the amount of these compounds also showed a significant increase and significant differences were observed between the different habitats. Also a significant positive relationship was observed between flavonoids and anthocyanins. The highest and lowest amount of Flavonoids and antocyanins observed in accessions 10 and 1 respectively.

***Corresponding Author:** Esmail Babakhanzadeh Sajirani ✉ esmailbabakhanzade@yahoo.com

## Introduction

*Echium amoenum* (Iranian GoleGavZaban) is a perennial endemic Iranian medicinal plant belonged to Boraginaceae family and naturally grown in mountainous regions of North of Iran. Four species of this genus are found in Iran, which *E. amoneum* is the only species in cultivation and consumption placed (Mozaffarian, 1996). The violet dry petals have been used as tonic, tranquillizer, diaphoretic, cough suppressant and a remedy for sore throat in folk Iranian edicine (Mehrabani *et al.,* 2005) Phytochemical investigation on this plant represents several chemical compounds, including anthocyanins (13%), flavonoid aglycones (15.%), saponins, unsaturated terpenoids, sterols and essential oils (0.05%) (Heidari *et al.,* 2006; Shafaghi *et al.,* 2002) and Pyrrolizidinealkaloids (Carlos *et al.,* 2013).

Anthocyanin pigments are responsible for attractive reddish purple and blue colors of many fruits and vegetables. Anthocyanins vessel have beneficial effects on heart disease, improve vision, antioxidant and anti-cancer activity Anti - are. Potential use in the food industry anthocyanins because being healthy and efficient, a lot of attention in the industry has led to (Wiley and Inc, 2001). Antioxidant properties of Anthocyanins have been reported in some studies (Sterling, 2000).

The amount of anthocyaninsis influenced by environmental factors such as light intensity, temperature, nutritional stress and pathogen attacks in plants. Intense light and low temperature conditions are favorable for the production of anthocyanins (Bourgaud *et al.,* 2001). Flavonoids Belongs to a group of natural polyphenols. Their main tasks of flavonoids are production of colored compounds such as chlorophyll and carotenoids. Have numerous applications in the food industry (Fiorucci, 2006). Over 5,000 flavonoids have been identified naturally in various plants (Harborneand Williams, 2000). This study is the first report about the measurement of flavonoids and anthocyanins in *Echium amoenum* in eshkevari accessions in North of Iran.

## Materials and methods

*Plant materials*

The petals of *Echiumamoenum*were collected from some accessions with different geographic characteristics in Eshkevari region, Guilan, in North of Iran during May and June 2013(table 1).

**Table 1**. Geographic positions of different accessions based on the GPS and Google earth software.

| Accession | Region name | Longitude(E) | Latitude(N°) | Altitude(m) |
|---|---|---|---|---|
| 1 | Milash | E50 12.933 | N36 53.641 | 693 |
| 2 | sajiran | E50 14.312 | N36 52.943 | 770 |
| 3 | Lima | E50 14.156 | N36 50.501 | 847 |
| 4 | Aghozbon | E50 12.544 | N36 52.835 | 858 |
| 5 | Kakroud | E50 16.133 | N36 48.256 | 1110 |
| 6 | Dargah | E50 20.447 | N36 43.256 | 1320 |
| 7 | Leshkan | E50 20.800 | N36 40.306 | 1772 |
| 8 | Baltorke | E50 23.146 | N36 43.391 | 1850 |
| 9 | Taklesh | E50 15.013 | N36 51.845 | 1966 |
| 10 | Siposht | E50 16.013 | N36 52.845 | 2125 |

*Total anthocyanins determination*

For measurement of total anthocyanin,1grpowdered petals of *Echium amoenum* were extracted with 50 ml of absolute methanol by maceration method for 24h in a mechanical shaker at room temperature. Extracts were filtered with a piece of filter paper (whatman No. 1) (Harbone, 1984).The total anthocyanin content was measured by the pH-differential method

described by Giusti and Wrolstad (2001) using 2 buffer systems: potassium chloride buffer with pH 1 and sodium acetate buffer with pH 4.5. The sample diluted with corresponding buffer and they were kept at room temperature for 15 min, the absorbance was measured at 510 and 700 nm. Total anthocyanins were calculated as cyanidin-3-glucoside according to the following equation:

TAC= (A × DF × MW × 100)/MA; TAC (Total anthocyanins), DF (dilution factor), MW (molecular weight), MA (molar absorptivity) and A= [(A510-A700) pH 1.0 - (A510-A700) pH 4.5].

*Total flavonoids determination*

Total flavonoids content of each extract was determined by aluminum chloride method (Pourmorad *et al.,* 2006). Plant extracts (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Quercetin was used as a standard for calibration curve. Total flavonoid values were expressed in terms of mg equal Quercetin in 1 g powder dry petals as plant. Results were reported as mg dry weight equivalents per gram qercitin.

Y= 7.939X*0.037; Y and X were spectrophotometer outputs and the amount of flavonoids respectively.

*Statistical analysis*

Data analyzed by SAS software (SAS Institute Inc., 2001). The ANOVA was performed for analysis of the data obtained for each experiment (P<0.01). The regression model was fitted to the data using the ProcReg of SAS.

**Results and discussion**

As shown in Table 2, the results indicated that the amount of anthocyanins and flavonoids were varied in different accessions and significant difference (P<0.01) was observed between them. Results of regression analysis and coefficient of determination showed that the anthocyanin content (R2 = 0.847) and flavonoids (R2 = 0.873) in different accessions have been severely affected by altitude. With increasing altitude, the amount of anthocyanins and flavonoids also showed a significant increase. The highest and lowest amount of flavonoids and anthocyanins was observed at accession 10 (N36 52.845 E50 16.013; 2125 m above sea level) and accession1 (N36 53.641 E5012.933; 693 m above sea level) respectively (Fig. 1 and 2). Moreover, our data showed a significant positive correlation between flavonoids and anthocyanins (0.96, P<0.01) (Table 3). Altitude and air temperature showed significant effects on anthocyanins and flavonoids content. The highest and lowest anthosyanin content were observed in 10 and 1 accessions, respectively. habitatas a factor affecting the accumulation of secondary metabolites has been emphasized (Hemmati *et al*., 2003). Location of plant growth can be affected the process of formation of secondary metabolites in plants through changes in temperature and humidity. The effect of cool air on the measured concentration of the anthocyanin and flavonoids can be linked to a longer period of cell division and plant tissue is in a cool area. Due to the high altitude, plants to cope with drought and temperature stress can synthesize large amounts of phenolic compounds. Considering the role of flavonoids in plant protection against UV light, higher-density flavonoids and anthocyanin in accession 10(2125 m above sea level) is justifiable.

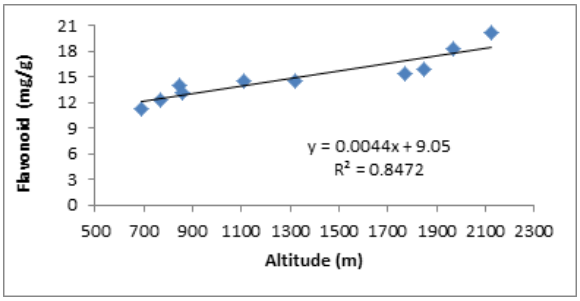


**Fig. 1.** Flavonoid content of *Echium amoenum* subjected to different altitudes.

**Table 2.** ANOVA of *Echium amoenum* phytochemical parameters estimate for the regression model relating the percentage significant difference of the mean and variance of different altitudes

| trait | RMSE | a ± SE | b ± SE | $R^2$ | CV% |
|---|---|---|---|---|---|
| **flavonoid** | 1.10 | 9.05 ± 0.95 ** | 0.004 ± 0.0006 ** | 0.847 | 7.35 |
| **anthocyanin** | 1.98 | 41.48 ± 1.71 ** | 0.008 ± 0.001 ** | 0.872 | 3.72 |

The parameter estimates are: RMSE = root *mean square error,* a= the intercept, b = the slope X, SE = standard error, $R^2$ = R Square, CV= *co-efficient* of variation, ** (P<0.01)

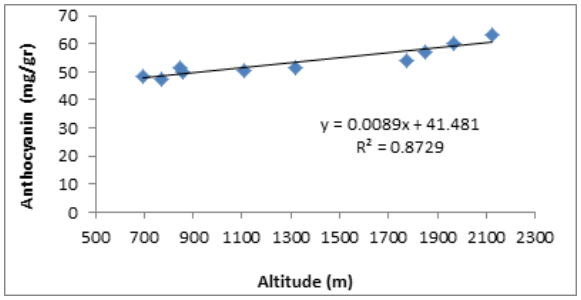


**Fig 2.** Anthocyanin content of *Echium amoenum* subjected to different altitudes.

**Table 3.** Correlation among some phytochemical traits of *Echium amoenum*.

| trait | flavonoid | anthocyanin |
|---|---|---|
| flavonoid | 1 | |
| anthocyanin | 0.96** | 1 |

** (P<0.01).

The Increasing amounts of flavonoids and anthocyanins in high altitudes are properties of the some plant's defense against ultraviolet rays. Increasing the concentration of flavonoids and antocyanins is due to the high activity of the PAL (Phenylalanine ammonialyse) or high speed synthesis of this enzyme under UV stress (Guo and Wang, 2008; Çiğdem and Ayşegül, 2011). Anthocyanins which are derived from phenolic compounds and through the flavonoid biosynthetic pathwaycanfilter the UV rays (Greenberg *et al.,* 1996).The high amounts of flavonoids and antocyanins in our study can be attributed to the increasing altitude and decreasing temperature.

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