Evaluation of Phytochemical and Production Potential of Borage (*Borago officinalis* L.) During the Growth Cycle

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Abstract

Background: Borage (*Borago officinalis* L.) is a valuable medicinal plant with a high content of gamma linolenic acid. It has an important place in Iranian traditional medicine. Although the aerial parts of this plant are extensively used in treatment of diseases, detailed knowledge of the active constituent changes in these parts is not readily available. In addition the most data that has been reported are about oil seed of borage and its properties.

Objective: Thus, the study about changes in chemical composition/secondary metabolites at different growth stages is necessary.

Method: For this reason, in the present research the chemical composition including total phenol, total alkaloids, mucilage, total ash, acid-insoluble ash and tannins of the aerial parts were evaluated during different growth stages of borage.

Results: Results indicated that at each corresponding stage of development, starting from seedling stage until flowering stage the chemical compositions of borage aerial parts hadn't significantly differences. Only, there were significant differences ($p<0.01$) in the total ash and acid-insoluble ash and these two parameters were significantly increased with the development of plants, from seedling until flowering stage. In general, at all stage, the aerial parts showed similar quality in respect of chemical compositions.

Conclusion: Therefore the best harvesting time of the aerial parts is the flowering stage, because we achieved the highest fresh and dry weight at this stage.

Keywords: *Borago officinalis* L., Aerial parts, Chemical Composition, Growth Stages

Introduction

Borage (*Borago officinalis* L.), is an annual herbaceous plant and native to Europe, North Africa, and Asia Minor [1]. It is an important vegetable crop which cultivated in some countries [2] including Iran. Also it is a medicinally important plant which has more than 20% gamma linolenic acid (GLA) in the seed oil [3]. The leaves of borage are reportedly used as diuretic, demulcent, emollient, expectorant, etc. [4]. In Iranian traditional medicine, the aerial parts of borage are reportedly used for treatment of a variety of ailments. Therefore, identification/determination chemical composition of aerial parts of this plant during growth cycle can be suggested the medicinal value of borage.

Chemical compounds such as amino acids, carbohydrates and proteins, are products of primary metabolism and are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids, are products of secondary metabolism and have toxicological, pharmacological and ecological importance [5]. However, the main classes of bioactive compounds from plants include flavonoids, terpenes, alkaloids, saponins, and coumarins [6].

Flavonoids are polyphenolic compounds [7] and the expression 'phenolic compounds' embraces a vast range of organic substances, which are aromatic compounds with hydroxyl substituents [5]. Plants phenolics compounds are economically important because: determining the sensory quality of foods such as color, taste, and flavor [5, 8], acquiring tolerance for aflatoxin contamination in peanut genotypes [9], protecting plants from herbivores[5], possessing biological activity in cancer and heart diseases prevention [10], acting as chemical signals in the flowering and pollination and in the process of plant symbiosis and parasitism [5], protecting organisms against the oxidative stress by their antioxidant activity [11], some possessing antibiotic properties[5], inhibit autoxidation of unsaturated lipids, preventing the formation of oxidized low-density lipoprotein (LDL), reducing cardiovascular disease [12] and to provide health-improving benefits due to their various biological activities such as antioxidative, anticarcinogenic, antimicrobial, and antimutagenic activity [8].

Alkaloids are nitrogenous bases (usually heterocyclic), and are structurally the most diverse class of secondary metabolites. They range from simple structures to complex ones such as those of many neurotoxins. Their manifold pharmacological activities have always excited man's interest, and selected plant products containing alkaloids have been used as poison for hunting, murder and euthanasia, as euphoriants, psychedelics, stimulants and medicine [5].

Tannins are polyphenolic substances widely distributed among higher plants [13, 14]. In addition, tannins have various uses in industry products such as manufacture of plastics, paints, ceramics and water softening agents. Increasing attention is also being paid to the use of tannins as antimicrobial agents (e.g. wood preservation) or prevention of dental caries. Recently, evidence has been provided in support of potential value of tannins as cytotoxic or antineoplastic agents [5]. However, tannin concentrations in plants vary in response to changes in environmental conditions [15].

Borage leaves contain up to 30% mucilage [4] which has medicinal value. Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums

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and those containing an appreciable amount of mucilage, pectin or hemicellulose [16].

The total ash includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant [16]. Also, total ash content can be indicated the total amount of salts in plant samples [17]. Content of acid-insoluble ash is the amount of silica present, especially as sand and siliceous earth which is present in plant sample [16, 17]. However, the content of total ash and acid-insoluble ash must not be more than extant of mentioned in herbal pharmacopoeias.

However, production of secondary metabolites, in general, is a highly ordered process with respect to plant development [18]. The concentrations of these compounds in plant tissues vary among plant parts and during the growing season. Therefore, determination the optimum harvesting time is important to obtain maximum natural production and assess the viability of a medicinal plant as a potential crop [19].

Despite a wealth of published research on borage seed oil, the variations of secondary metabolites at different growth stage aren't well documented. Thus, it is necessary to study changes of the secondary metabolites at the different growth stage. For this reason, this study was conducted in order to determining the changes that might take place in fresh and dry matter and the chemical composition in aerial parts of *Borago officinalis* L. at different growth stage.

Methods and Materials

Plant materials

An experiment was conducted in plant science laboratory of Tarbiat Modares University, Iran, from September 2006 to November 2007. Seeds of *Borago officinalis* L. were obtained from the Institute of Medicinal Plants Research of Iranian Academic Centre for Education, Culture & Research (ACECR). Seeds were selected for uniformity in size, shape, and color. The soil of pots was a noncalcareous sandy loam containing 54% sand, 30% silt and 16% clay. The available soil water between wilting coefficient and field capacity ranges from 6.0% to 14.7%, respectively. The total organic carbon content was 0.3% and pH of soil was 7.3. Manuring, irrigation and other field practices had been done as needed.

This experiment was laid out in a completely randomized design with three replicates to study changes of the secondary metabolites at the different growth stages (including: seedling, vegetative and flowering stages). The aerial parts at seedling (fourteen-day-old), vegetative (start of plant stem elongation) and flowering (50% flowering) stages were harvested and used to determine the growth and chemical compositions. At each growth stage, from each replicate, ten plants were sampled to determine dry weight (DW) and fresh weight (FW). The plants materials were oven dried at 70 ◦C for 72h (until there was no decrease in weight).

Biochemical analysis

Total phenolic contents were determined using chlorogenic acid as a standard molecule with the Folin-Dennis method, as described by Iranian Herbal Pharmacopeia (2002). The results were expressed as the chlorogenic acid equivalent for the total polyphenols.

Total alkaloids were quantified from the dried shoots tissues of the plants in base of kelidonin as described by Iranian Herbal Pharmacopeia (2002).

Tannins were quantified using tannic acid as a standard mulecule with the Folin-Dennis

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method, as described by Iranian Herbal Pharmacopeia (2002).

The swelling index was the volume in ml taken up by the swelling of 1 g of plant material under specified conditions. This index was determined with the method which described by WHO (1998).

Mucilage content was quantified as described by Iranian Herbal Pharmacopeia (2002).

The ash remaining (following ignition of medicinal plant materials) was determined by different methods. In this study, the total ash and acid-insoluble ash of the aerial parts was determined according to method of Iranian Herbal Pharmacopeia (2002).

Statistical Analysis

Analysis of variance (ANOVA) was done by SPSS statistical software package version 12. Mean values and significance were determined by "Duncan's multiple range test".

Results

Analysis of variance for different parameters is shown in Table 1. There weren't significant differences ($p< 0.05$) in the chemical composition (total phenol, total alkaloids, mucilage and tannins) and the swelling index among three different growth stages (Tab.1 and 2). The mean of total phenol, total alkaloids, mucilage, tannins, swelling index and ratio of mucilage/swelling index at three stages were 4.32%, 7.8ppm, 10.96% 2.93%, 14.16 and 1:1.29, respectively.

In contrast to chemical composition, there were significant differences ($p<0.01$) in fresh and dry weight, total ash and acid-insoluble ash (Tab.1). The fresh and dry weight of aerial parts were significantly increased during the growth cycle from 0.102 and 0.0.01gr/plant at the seedling to 42.07 and 4.38 gr/plant at the flowering stage respectively (Tab. 2).

As table 2 indicated, pattern of increasing in total ash and acid-insoluble ash content during growth stages was similar with pattern of fresh and dry accumulation. In other words, total ash and acid-insoluble ash were significantly increased from seedling to flowering stage. The total ash and acid-insoluble ash content were increased from 6.78 and 1.06 percent at seedling stage to 8.60 and 1.31 percent at flowering stage, respectively.

Table 1. Mean squares from analyses of variance of data for measured attributes of *B. officinalis* at different growth stages.

Source of variation	df			
		Fresh weight	Total alkaloids	Mucilage
Treatments	2	1324.693***	0.043 $^{n.s}$	0.0404 $^{n.s}$
Error	6	5.567	0.212	0.282
		Dry weight	Tannins	Total ash
Treatments	2	14.316***	0.006 $^{n.s}$	2.236**
Error	6	0.051	0.013	0.159
		Total phenol	Swelling index	Acid-insoluble ash acid
Treatments	2	0.214 $^{n.s}$	1.254 $^{n.s}$	0.065***
Error	6	0.128	0.319	0.002

n.s: non-significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 2. Chemical composition, fresh weight and dry weight of borage at three stages of growth cycle

Growth Stage	Fresh weight (g)	Dry weight (g)	Total phenol (%)	Total alkaloids (ppm)	Tannins (%)	Swelling index	Mucilage (%)	Total ash (%)	Acid-insoluble ash (%)
Seedling	$0.102^{c}\pm0.0024$	$0.010^{c}\pm0.0002$	$4.067^{a}\pm0.1764$	$7.833^{a}\pm0.2028$	$2.883^{a}\pm0.0833$	$13.733^{a}\pm0.3283$	$11.333^{a}\pm0.3383$	$6.873^{c}\pm0.1899$	$1.056^{b}\pm0.0328$
Vegetative	$22.996^{b}\pm1.0182$	$2.131^{b}\pm0.1259$	$4.600^{a}\pm0.2309$	$7.667^{a}\pm0.3283$	$2.970^{a}\pm0.0351$	$14.900^{a}\pm0.3215$	$10.933^{a}\pm0.2906$	$7.733^{b}\pm0.2728$	$1.310^{a}\pm0.0264$
Flowering	$42.071^{a}\pm2.1284$	$4.378^{a}\pm0.1874$	$4.300^{a}\pm0.2082$	$7.900^{a}\pm0.2517$	$2.943^{a}\pm0.0722$	$13.833^{a}\pm0.3283$	$10.600^{a}\pm0.2887$	$8.600^{a}\pm0.2205$	$1.313^{a}\pm0.0260$

Discussions

Many studies on medicinal plants indicated that chemical content composition of these plants may vary substantially with the developmental stage of the plants. For this reason, investigations on ontogenetic variation of secondary metabolites from different classes have received considerable interest from plant scientists over several decades [20]. In the present study, ontogenetic changes in total phenol, total alkaloids and mucilage of plant materials weren't found to be significant ($p > 0.05$) and only the plant material had significant differences ($p < 0.01$) in respect of biomass accumulation per each plant (fresh and dry weight), total ash and acid-insoluble ash content. Therefore, Plants produced similar amount of secondary metabolites at all stages of plant phenology. In other words, the aerial parts of borage at the different growth stages have great pharmaceutical potential, with their well documented phenolic compounds, tannins, mucilage and alkaloids content. Of course, the highest yields of secondary metabolites in whole shoots (aerial parts) were reached at flowering time because the highest yields of shoots obtained at this stage while the amount of secondary metabolites was similar at different growth stage (Tab. 1 and 2).

The present study indicated that total phenol of borage leaves was approximately 4.32% of dry mater which was noticeable. The pervious study [21] shown that rosmarinic acid, syringic acid and sinapic acid are the major phenolic compounds present in the ethanolic extract of borage meal. These compounds account for approximately 4 and 0.6% of the dry mass of the crude extracts and the defatted seeds, respectively. Also, Wettasinghe and Shahidi (2002) reported that the total phenolics content of borage meal was 413 mg/g [22].

Although the contents of secondary metabolites were statistically similar at the different growth stages, to have more complete information about this plant and its potential uses, further studies could be conducted on borage shoot at different stages. In other words, additional studies are needed to investigate recognition /identification bioactive constituents at different stages.

In conclusion, this study indicated that the flowering stage of borage was the optimal harvesting period to reach maximize yield of secondary metabolites due to the highest fresh and dry weight of aerial parts at this stage.

References

1. Beaubaire NA and Simon JE. Production potential of borage (*Borago officinalis* L.) *Acta Hort*. 1987; 208: 101.

2. Montaner C, Floris E, and Alvarez JM. Is self-compatibility the main breeding system in borage (*Borago officinalis* L.). *Theor. Appl. Genet*. 2000; 101: 185 – 9.

3. EL Hafid RE, Blade SF and Hoyano Y. Seeding date and nitrogen fertilization effects on the performance of borage (*Borago officinalis* L.). *Industrial Crops and Products*. 2002; 16: 193 - 9.

4. Leung AY and Foster S. Encyclopedia of common natural ingredients- Used in food, drugs and cosmetics.2nd ed. A Wiley-Interscience Publication. USA. 1996, pp: 98 -9.

5. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management*. 2002; 10**:** 421 – 52.

6. Cowan, M. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*. 1999; 12: 564 – 82.

7. Hrazdina G. 'Anthocyanins,' in J.B. Harborne and T. J. Mabry (eds.), *The Flavonoids: Advances in Research*, Chapman and Hall Ltd, Cambridge, Great Britain, 1982; pp: 135 – 89.

8. Kim DO. Cherry phenolics and their protective effects on neuronal cells. The 89th International Symposium of the KSABC, Gwangiu- Korea. 2006, p: 88.

9. Latha P, Sudhakar P, Sreenivasulu Y, Naidu PH and Reddy PV. Relationship between total phenols and aflatoxin production of peanut genotypes under end-of-season drought conditions*. Acta Physiol. Plant*. 2007; 29: 563 – 6.

10. Kris-Etherton PM, Hecker KD, Bonamone A, Coval SM, Binkoski AE, Hilpert KF, Griel AE and Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* 2002; 113: 71S – 88S.

11. Bravo L. Polyphenols: chemistry, dietary sources,metabolism, and nutritional significance. *Nutr. Rev.* 1998; 56: 317 – 33.

12. Oboh G. Antioxidant properties of some commonly consumed and underutilized tropical legumes. *Eur. Food Res. Technol*. 2006; 224: 61 – 5.

13. Kraus TEC, Dahlgren RA and Zasoski RJ. Tannins in nutrient dynamics of forest ecosystems - a review. *Plant and Soil*. 2003; 256: 41 – 66.

14. Ferwerda JG, van Wieren SE, Skidmore AK and Prins HHT. Inducing condensed tannin production in *Colophospermum mopane*: Absence of response to soil N and P fertility and physical damage. *Plant and Soil*. 2005; 273: 203 – 9.

15. Chaves N and Escuder JC. Variation of flavonoid synthesis induced by ecological factors. *In* Principles and Practices in Plant Ecology – Allochemical Interactions. Eds. Inderjit KMM Dakshini and CL Foy. CRC Press, Boca Raton. 1999, pp: 267 – 85.

16. WHO. Quality control methods for medicinal plant materials. World Health Organization, Geneva. 1998.

17. Iranian Herbal Pharmacopoeia (IHP). Tehran: Ministry of Health Publication, volume1. 2002.

18. Dutta A, Batra J, Pandey-Rai S, Singh D, Kumar S and Sen J. Expression of terpenoid indole alkaloid biosynthetic pathway genes corresponds to accumulation of related alkaloids in *Catharanthus roseus* (L.) G. Don. *Planta*. 2005; 220: 376 – 83.

19. Taylor JLS and Staden J. The effect of age, season and growth conditions on anti-inflammatory activity in *Eucomis autumnalis* (Mill.) Chitt. Plant extracts. *Plant Growth Regulation.* 2001; 34: 39 – 47.

20. Cirak C, Radusiene J, Ivanauskas L and Janulis. Variation of bioactive secondary metabolites in *Hypericum origanifolium* during its phenological cycle. *Acta Physiol Plant*. 2007; 29: 197 – 203.

21. Wettasinghe M, Shahidi F, Amarowicz R and Abou-Zaid MM. Phenolic acids in defatted seeds of borage (*Borago officinalis* L.). *Food Chemistry* 2001; 75: 49 – 56.

22. Wettasinghe M and Shahidi F. Iron (II) chelation activity of extracts of borage and evening primrose meals. *Food Research International*. 2002; 35: 65 – 71.

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