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The Chemical Composition Profile of *Dorystoechas hastata*Boiss. & Heldr. Ex Bentham Cultivatedin Turkey

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Abstract:*Dorystoechas hastata*Boiss. and Heldr. Ex Benthamis a monotypic plant endemic to Antalya province of Turkey. The chemical compositions of the water distilled essential oil of *D. hastata* different parts were investigated by GC/MS, which cultivated in Konya, Turkey. Three major compounds were identified from the aerial parts of *D. hastata.* Guaiol was the main component with 26.5 % of the branch essential oil. The leaf essential oil contained20.6 % 1,8-cineole, and the aerial parts contained15.0% borneol, respectively. Additionally, *D. hastata*cultivated samples were investigated for their macro (P, K, Ca, Mg, Na), micro (Fe, B, Mn, Zn, Cu) and heavy metal (Pb, Cd, Ni, Cr, Co) contents. The phosphorus (P) contents of *D. hastata* leaf, branch and the aerial parts were found as 3164, 2441 and 1852 ppm, respectively. Also, the amino acid compositions of different parts of *D. hastata* were investigated by HPLC. Major component of proteins in three different part of the plant was identified as proline. The highest proline content of *D. hastata* was found, however in the branch as 47.1 mg/100 g. As a conclusion, wihin this present study detailed chemical content of the cultivated *D. hastata* was investigated for the first time respective to its various parts.

Keywords:*Dorystoechas hastata*; essential oil; minerals and amino acids.

1. Introduction

Medicinal and aromatic plant species are one of the most important sources of food and medicines for humans as well as for feed for animals. In the last decades, increasing attention to natural products and unreasonable harvesting activities have led to an increase in the existing pressure and danger on natural plant species [1]. Many wild plants are spread endemically and being consumed by local inhabitants traditionally as tea, flavorings or spice*etc*. [2]. *Dorystoechas hastata* Boiss. and Heldr. Ex Bentham (Lamiaceae) is a wild monotypic plant endemic to the Southwest Anatolia, Turkey [3]. *D. hastata* is an perennial medicinal and aromatic plant. Fresh or dried *D. hastata* leaves are used to make an aromatic tea, locally known as "şalba çayı" (= chalba tea), with a pungent taste, which is used as a healing beverage against common cold or as a health drink by the local inhabitants [4]. Many of the folk medicine in the Mediterranean subdivision of Turkey are investigated and extensive use of plants belonging to Lamiaceae family as a tea, as a tonic or as remedies forvariousdisordersisreported [5, 6].

The products are valuable as dietetics due to the mineral and element compositions. Several nutritionally important macro and micro trace elements (P, K, Ca, Mg, Na, Fe, B, Mn, Zn and Cu) constitute an important part of the composition [7]. The role of neurotransmitter amino acids in the function of the nervous system has been the focus of increasingly intense research over the past

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several years. The most studied amino acids are glutamate (Glu), aspartate (Asp), gamma-aminobutyric acid (GABA), and glycine (Gly). Glutamate and aspartate are the main excitatory amino acids in the brain and are responsible for normal synaptic neurotransmission, while gamma amino butyric acid and glycine are inhibitory; GABA is the major inhibitory neurotransmitter in the mammalian brain [8]. As with many of the Lamiaceae family, essential oils of *D. hastata* have been investigated, and 1,8-cineole, α-pinene, borneol, guaiol and camphor have been found to be the main compounds of its essential oils [9]. In another study, diterpenes and norditerpenes from its roots were described [10]. Additionally, It was studied the proline and antioxidant contents of the leaves and showed that the species may serve as a natural source of proline and antioxidants [4]. It was reported that *D. hastata* can be used as a potential antioxidative edible source due to its different classes of phenolic compounds and strong antioxidative capacity[11].

To the best of our knowledge, in spite of numerous studies of the species of the family Lamiaceae, there is no previouslypublished study on the cultivated *Dorystoechas hastata* and its detailed chemical compositionsuch as elemental analyses, composition of essential oil, and amino acid profile, respectively. Thus the aim of the present study was to determine the variation of the essential oil, elemental and amino acid composition of *D. hastata* cultivated in Turkey.

2. Materials and Methods

2.1. Chemicals and Materials

Ethyl alcohol, diethyl ether, HCl, NaOH, methanol, glacial acetic acid, acetonitrile were purchased from Merck, Germany. Borate buffer solution (BBS), 9-fluorenylmethylchloroformate (FMOC) reagent, o-phthalaldehyde and 3-mercaptopropionic acid (OPA) reagent, 10, 25, 100, 250, 1000 pmol/mL internal amino acid standards (aspartic acid, glycine, alanine, valine, leucine, isoleucine, serine, threonine, tyrosine, proline, arginine, histidine, glutamic acid, cystine, phenylalanine, lysine, methionine) and external amino acid standards (norvaline, sarcosine, asparagine, glutamine, tryptophane and hydroxyproline) were obtained from Agilent Co., Switzerland. Phenol was from Sigma-Aldrich, Germany. All other chemicals and solvents were of analytical grade,which were purchasedfrom Turkey. Deionized double distilled water was used in all the experiments.

2.2. Plant Material

The wild-grown samples of *Dorystoechas hastata* were collected during the flowering period from mountain region (1500 m from the sea level) of Antalya, Turkey in June, 2010. Taxonomic identification was performed by botanists in the Biology Department, Faculty of Science, Selçuk University. The seeds of the plant were collected from full matured period in September, 2010, for cultivation. The seeds were cultivated in the experimental farm (1000 m from the sea level) at Selçuk University, Konya, Turkey, in March, 2011. The cultivated samples of *D. hastata* were harvested in June, 2012. The voucher specimen was preserved at the Herbarium of the Faculty of Agriculture, Selçuk University, Konya(Voucher No: TAB 0102).

2.3. Essential Oil Distillation

The air-dried different aerial parts of *Dorystoechas hastata* were subjected to water distillation for 3 h using a Clevenger-type apparatus to produce essential oil (yields were given in Table 1.). The obtained essential oil was stored at +4°C until the analyses.

2.4. Gas chromatography/mass spectrometry (GC/MS)

The GC/MS analysis was carried using Agilent 6890N system combined with Agilent 5973 GC/MSD system. DB Wax column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium

as carrier gas (1.2 mL min^{-1}). GC oven temperature was kept at 60 ºC for 10 min and programmed to 220 ºC at a rate of 4 ºC min^{-1}, and kept constant at 220 ºC for 10 min and then programmed to 240 ºC at a rate of 1 ºC min^{-1}. Split ratio was 40:1. The injector temperature was 250 ºC. MS were obtained at 70 eV, and the range was from *m/z* 35 to 450. The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 ºC. To obtain the same elution order with GC/MS, simultaneous auto-injection was performed duplicatedusing the same column having the same conditions. Relative percentage amounts (%) of the separated compounds were obtained from the FID chromatograms. Identification of the essential oil components was performed by comparison of their relative retention times with those of authentic samples or by their relative retention index (RRI) to series of *n*-alkanes (C6–C24). Computer matching against commercial libraries (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) [20] as well as MS literature data [21] was used for the identification.

2.5. Elemental Analyses

The macro (P, K, Ca, Mg, Na), micro (Fe, B, Mn, Zn, Cu) and heavy metal (Pb, Ni, Cr, Co contents were determined by using various techniques. P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, B, Mn, Cu, Zn, Al, Pb, Ni, Cr and Co was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with statistical data comparison to a certified reference plant material, respectively [19].

2.6. Hydrolysis of Amino Acids

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. Hydrolysis Solution was 6 N hydrochloric acid containing 0.1-1 % of phenol. The branch, leaf and herb of *Dorystoechas hastata* were placed in a hydrolysis tube and dried. The samples were dried so that water in the sample would not dilute the acid used for the hydrolysis. Samples were typically hydrolyzed at 110ºC for 24 hours in vacuum or inert atmosphere to prevent oxidation. After hydrolysis, samples were dried in vacuum to remove any residual acid. The hydrolysates were filtered and injected to HPLC [12,13].

2.7. High-Performance Liquid Chromatography (HPLC)

The analysis was performed with a system consisting of HP Agilent 1200 series Quaternary Pump with degasser, injector and photodiode array detector. Samples were injected with a HP Agilent 1200 Autosampler with thermostatic column compartment on a Zorbax 200 × 2.1 mm column and guard column Hypersil ODS(20 × 2.1). The DAD (diode array detector) detector was set at 338/10 nm and 262/16 nm, flow rate 0.5 mL/min and oven temp: 40 ºC. System was controlled and data analyses were performed with Agilent ChemStation. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

2.8. Statistical Analysis

Each parameter was tested in triplicates with independent three replications. Conventional statistical methods were used to calculate means. Collected data were subjected to statistical analyses using JMP statistical package software (Version 5.0.1.a, SAS Institute. Inc.Cary, NC). One way ANOVA was used to evaluate the effect of the plant part (branch, leaf and whole aerial parts) on the parameters studied. When significant ($p< 0.01$) main effect was found, the mean values were further analyzed using t test.

3. Results and Discussion

Dorystoechas hastata is a well-known monotypic endemic plant, which makes it difficult to compare its contents with another plant from the same genus.

3.1. The Essential oil Composition of Dorystoechas hastata

Table 1. Summarizes the results of essential oil compositions from different parts of*Dorystoechas hastata*. The oils of dried different parts of *D. hastata* were obtained by water distillation, with yields of 0.6 % for branch, 1.8 % for leaf and 1.5 % for whole aerial parts *D. hastata* (see also Table 1. as well as Figure 1.). In the course of the present study,33 components amounting to 85.9-97.7 % of the oils were identified in two different (branch and leaf) and whole aerial parts of *D. hastata.* The major components of branch, whole aerial parts and leaf oil were 26.5 % guaiol (champacol), 16.6% 1,8-cineole and 20.6% 1,8-cineole, respectively (Figure 2.). Comparison of the three different parts of *D. hastata* oil samples has shown that the 1,8-cineole content of the leaf oil was higher than those of branch and herb samples examined. The major compounds of the essential oil of *D. hastata* branch, whole aerial parts and leaf were found to be 10.2% and 15 % borneol as well as % 16.4 camphor, respectively. Baser and Ozturk reported [9] that forty-seven compounds representing 87–98 % of the oil *D. hastata* were identified. When compared, the essential oil compositions varied according to the distillation method, plant part and collection site. Major components were identified as 1,8-cineole, α-pinene, borneol, guaiol and camphor. The results of our analyses were in agreement and consistent with those reported previously by Baser and Ozturk [9]. Our results showed that, observed differences in the composition and yield of the oils appear to be climatic, seasonal, geographic [14], soil and other cultivation conditions [15], as well as the part of plant and the harvest period [16].

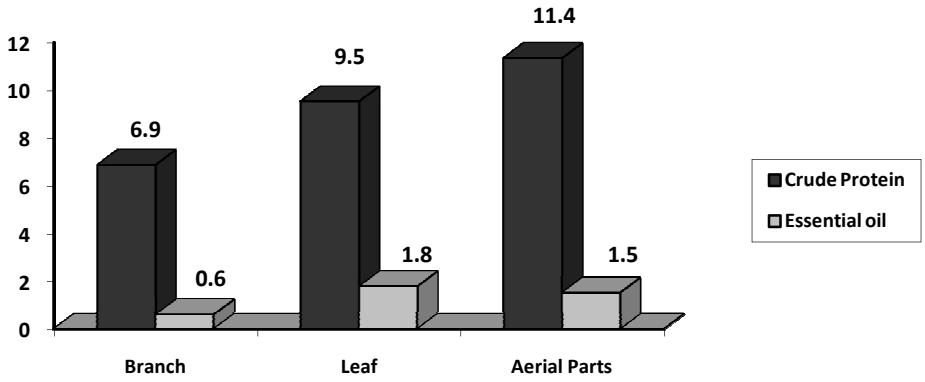

Figure 1. Crude protein and essential oil contents of *Dorystoechashastata*cultivated in Turkey

Table 1. Essential oil compositions of different parts of *Dorystoechashastata* cultivated in Turkey

RRI*	Compounds (%)	Branch	Leaf	Whole Aerial Parts
1023	**α-pinene**	**0.8^{c}**	**7.0^{b}**	**8.3^{a}**
1026	α-thujene	t	0.2^{a}	0.2^{b}
1067	camphene	0.6^{c}	4.7^{b}	5.4^{a}
1113	**β-pinene**	**1.2^{c}**	**8.7^{b}**	**9.7^{a}**
1150	γ terpinene	t	0.2^{ab}	0.5^{a}
1151	δ-3-carene	t	0.3^{a}	t
1165	myrcene	1.2^{c}	7.9^{b}	8.2^{a}
1179	δ-2-carene	t	0.1^{ab}	0.2^{a}
1198	limonene	0.5^{c}	2.7^{b}	2.7^{a}
1204	**1,8-cineole**	**5.3^{c}**	**20.6^{a}**	**16.6^{b}**
1210	*trans*-2-hexenal	t	0.2	t
1217	*trans*-β-ocimene	t	0.3	0.3
1223	γ-terpinene	t	0.2^{a}	0.3^{a}
1237	*p*-cymene	t	0.1^{b}	0.3^{a}
1243	terpinolene	t	0.7^{a}	0.5^{b}
1441	1-octen-3-ol	t	0.8^{a}	0.6^{b}
1459	*trans*-sabinene hydrate	0.1^{a}	0.2^{a}	t
1526	**camphor**	**9.9^{c}**	**16.4^{a}**	**14.2^{b}**
1540	linalool	0.4^{b}	0.6^{a}	0.5^{ab}
1583	bornyl acetate	3.8^{a}	1.8^{b}	2.1^{b}
1606	**β-caryophyllene**	**8.7^{a}**	**2.7^{a}**	**4.6^{a}**
1675	δ-terpineol	t	0.2^{a}	0.2^{a}
1683	α-humulene	1.2^{a}	0.6^{ab}	0.3^{b}
1697	γ-cadiene	0.3	t	t
1700	α-terpineol	0.3^{a}	0.5^{a}	0.5^{a}
1711	**borneol**	**10.2^{c}**	**16.4^{a}**	**15.0^{b}**
2006	caryophyllene oxide	3.2^{a}	0.2^{c}	0.4^{b}
2099	**guaiol (=*champacol*)**	**26.5^{a}**	**3.0^{c}**	**5.3^{b}**
2114	α-gurjunene	2.3^{a}	tr	t
2137	spathulenol	4.3^{a}	0.3^{b}	0.2^{c}
2138	α-eudesmol	1.1^{a}	t	0.2^{b}
2147	β-eudesmol	1.2^{a}	t	0.2^{b}
2487	abietatriene	2.6	t	t
	TOTAL (%)	**85.9**	**97.7**	**97.5**
	Essential oil yield(%)	0.6	1.8	1.5

Common letters in the same column indicate significant differences between the means at $p<0.01$ level.
$^{a,b,c,\ ab}$ represent statistical difference between the figures
*RRI: Relative retention index, t:trace

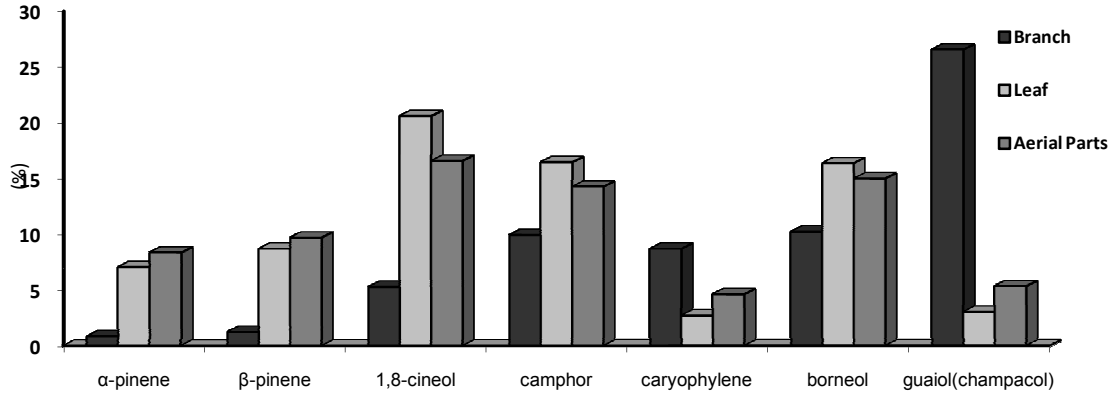

Figure 2. Major essential oil components of *Dorystoechashastata* cultivated in Turkey

3.2. The Mineral Composition of Dorystoechas hastata

The most challenging aspect of providing trace elements in plant-based material is to obtain a sufficient concentration for the supplements to be ingested without consuming large quantities of plant tissue. Table 2.represents the mean value of the analysed macro (P, K, Ca, Mg, Na) and micro trace elements (Fe, B, Mn, Zn, Cu) and heavy metal (Pb, Ni, Cr, Co) in different aerial parts of *Dorystoechas hastata*.

The most abundant minerals in*Dorystoechas hastata* were potassium (24166 ppm) in the branch,phosphorus (3164 ppm), magnesium (4115 ppm) and calcium (26832 ppm) in the leaf and sodium (210 ppm) in the whole aerial parts (Figure 3.), while iron (568 ppm), boron (151 ppm), manganese (139 ppm), zinc (41 ppm) and copper (68 ppm) were in minor amount in the leaf (Figure 4.). The heavy metals (Pb, Ni, Cr, Co) were in very low amounts (Figure 5.). The macro and micro element contents leaves of *D. hastata* were higher than branch and aerial parts. To the best of our knowledge, this is the first report on micro, macro elements and heavy metals of cultivated *D. hastata*.Several factors may affect the elemental contents of plants such as the variety, harvesting time, soil type, soil conditions, fertilization, irrigation and weather etc. [17-19]. This study shows a comprehensive presentation of macro and micro elements of *D. hastata s*amples cultivated in Turkey. According to the results, it can be concluded thatthe elemental composition and the nutritional value of *D. hastata* is worthwhile to investigate with comparison to other Lamiaceaefamily species used medicinally.

Table 2. Elemental composition of different parts of *Dorystoechashastata*cultivated in Turkey

	Macro Elements (ppm)					**Micro Elements (ppm)**					**Heavy Metals (ppm)**			
Plant Part	**K**	**Ca**	**Mg**	**P**	**Na**	**Fe**	**B**	**Mn**	**Zn**	**Cu**	**Pb**	**Ni**	**Cr**	**Co**
Branch	**24166**^{a}	12800^{b}	1720^{b}	2441^{b}	155^{b}	85^{c}	74^{c}	29^{c}	25^{c}	10^{b}	**3.6**^{a}	1.0^{c}	0.7^{b}	0.1^{c}
Leaf	18754^{b}	**26832**^{a}	**4115**^{a}	**3164**^{a}	11^{c}	**568**^{a}	**151**^{a}	**139**^{a}	**41**^{a}	**68**^{a}	2.9^{b}	1.6^{b}	1.7^{a}	**0.6**^{a}
Whole Aerial Parts	18087^{c}	13118^{b}	2167^{b}	1852^{c}	**210**^{a}	417^{b}	84^{b}	68^{b}	32^{b}	9^{c}	0.6^{c}	**2.6**^{a}	**1.8**^{a}	0.2^{b}

Common letters in the same column indicate significant differences between the means at $p<0.01$ level.
^{a,b,c} represent statistical difference between the figures

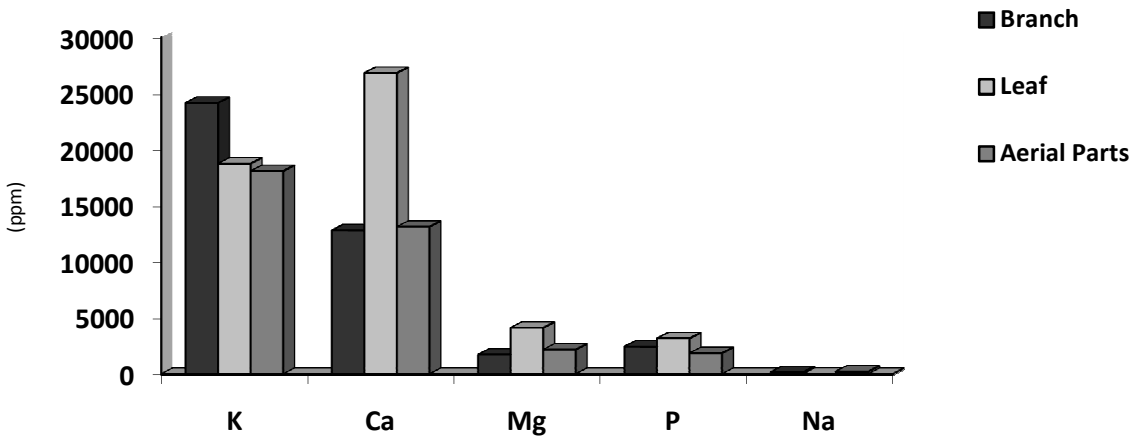

Figure 3. Comparative macro element compositions of *Dorystoechas hastata* cultivated in Turkey

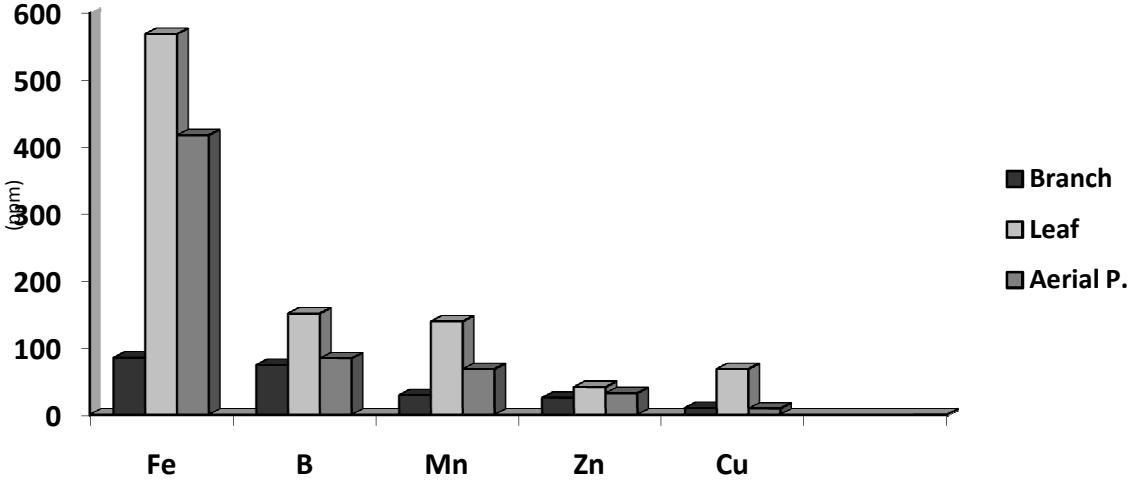

Figure 4. Comparative micro element compositions of *Dorystoechas hastata* cultivated in Turkey

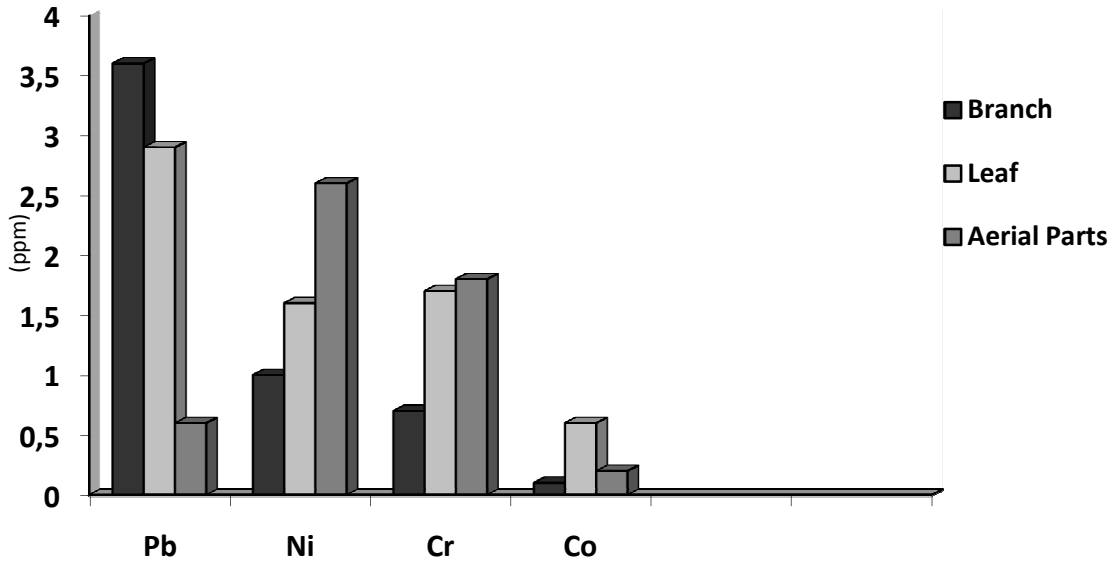

Figure 5. Heavy metal compositions of *Dorystoechas hastata* cultivated in Turkey

3.3.The Amino Acid Profile of Dorystoechas hastata

In this present study, the protein yields of dried different part of *Dorystoechas hastata* were 6.9 % for branch, 9.5 % for whole aerial parts and 11.3 % for leaf as shown in Figure 1.Twelve components of the non-essential amino acid were identified in three different aerial part of *D. hastata* as seen in Figure 6. The results of non-essential aminoacid compositions of *D. hastata* cultivated in Turkey was given in Table 3. The major components of branch, whole aerial parts and leaf protein were proline and 47.1, 45.9 and 35.7mg/100g, respectively (see also Figure 7). It was previously reported that *D. hastata* extracts contained relatively high levels of proline [4]. The results presented in this study,suggest that *D. hastata* may provide a natural source for the protein proline from plants. Other interesting amino acids found in the plant material were sarcosine (15.6 mg/100g), hydroxyproline (4.5 mg/100g), glutamine (2.6 mg/100g), glutamic acid (1.2 mg/100g) and alanine (1.0 mg/100g) in branch plant material. The results, as seen in Table 4.,summarize the essential aminoacid compositions obtained from*D. hastata* protein extract(Figure. 6). Essential aminoacid compositions of *D. hastata* branch proteins were found to possess higher than those of whole aerial parts and leaf amount of threonine (2.8 mg/100g), arginine (1.5 mg/100g) and tryptophan (0.8 mg/100g). To the best of our knowledge, this is the first report on the compositions amino acid of cultivated *D. hastata* along with detailed chemical analyses of the other parts of the plant.

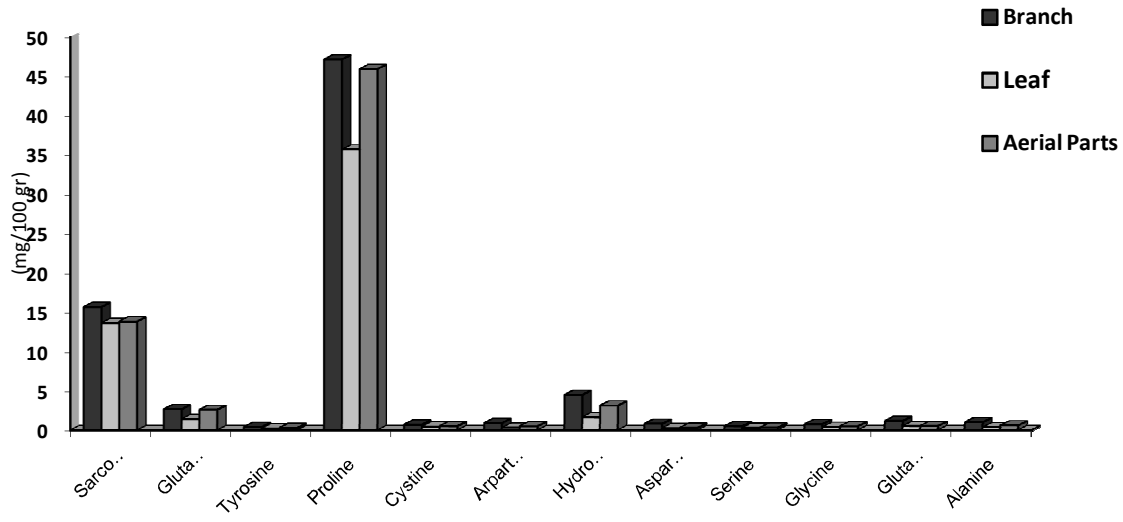

Figure 6. Non-essential aminoacid compositions of *Dorystoechas hastata* cultivated in Turkey

Table 3. Non-essential aminoacid compositions of *Dorystoechas hastata* cultivated in Turkey

	Non-essential aminoacid (mg/100 g)											
Plant Part	Sarcosine	Glutamine	Tyrosine	Proline	Cystine	Aspartic acid	Hydroxyproline	Asparagine	Serine	Glycine	Glutamic Acid	Alanine
Branch	**15.6**^{a}	**2.6**^{a}	**0.4**^{a}	**47.1**^{a}	**0.7**^{a}	**0.9**^{a}	**4.5**^{a}	**0.8**^{a}	**0.5**^{a}	**0.8**^{a}	**1.2**^{a}	**1.0**^{a}
Leaf	**13.6**^{a}	1.4^{b}	0.2^{b}	**35.6**^{b}	0.4^{b}	0.3^{c}	1.6^{b}	0.2^{b}	0.3^{b}	0.4^{c}	0.5^{b}	0.3^{b}
Whole Aerial Parts	**13.8**^{a}	2.6^{a}	0.3^{ab}	**45.9**^{a}	0.5^{b}	0.5^{b}	3.1^{a}	0.3^{b}	0.3^{b}	0.5^{b}	0.5^{b}	0.6^{ab}

Common letters in the same column indicate significant differences between the means at $p<0.01$ level.
$^{a,b,c,\,ab}$ represent statistical difference between the figures

Table 4. Essential amino acid compositions of *Dorystoechashastata* cultivated in Turkey

	Essential amino acids (mg/100 g)									
Plant Part	Arginine	Isoleucine	Lysine	Phenylalanine	Tryptophan	Histidine	Leucine	Methionine	Threonine	Valine
Branch	**1.5**^{a}	**0.3**^{a}	**0.4**^{a}	**0.5**^{a}	**0.8**^{a}	**0.3**^{a}	**0.6**^{a}	**0.6**^{a}	**2.8**^{a}	0.2^{b}
Leaf	0.3^{b}	0.2^{c}	0.3^{b}	0.2^{b}	0.2^{c}	0.3^{b}	0.4^{b}	0.3^{b}	**1.1**^{b}	0.3^{a}
Whole Aerial Parts	0.4^{b}	0.3^{b}	0.3^{b}	0.3^{b}	0.3^{b}	0.3^{b}	0.4^{b}	0.2^{c}	**1.2**^{b}	**0.3**^{a}

Common letters in the same column indicate significant differences between the means at $p<0.01$ level.
$^{a,b,c,\,ab}$ represent statistical difference between the figures

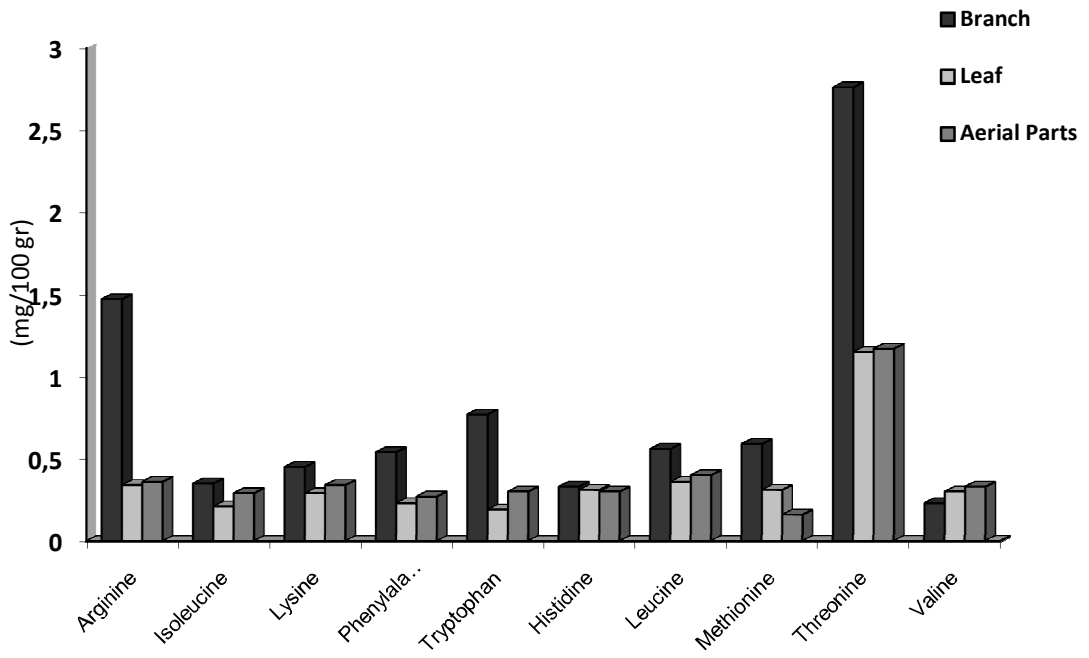

Figure 7. Essential aminoacid compositions of *Dorystoechas hastata* cultivated in Turkey

As an overallconclusion,*Dorystoechas hastata* cultivated in Turkeyhas relatively high levels of essential oil, minerals and amino acids as phyto-constituents. As a result, *D. hastata* can be used as natural ingredient source in pharmaceutical and food processing including veterinary applications. These results will contribute to further investigations for sources of other phytochemical substances from *D. hastata*.

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