
Araştırma Makalesi / Research Article

The Determination of Lipide- Soluble Vitamin Contents of Some *Astragalus* Taxa by Using HPLC

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Abstract

The goal of current study is to contribute the biochemical studies of five *Astragalus* (*Astragalus anthlloides*, *Astragalus hirsutus*, *Astragalus campylorhynchus*, *Astragalus cephalotes* var. *cephalotes*, *Astragalus odoratus*) species by determining the lipide-soluble vitamin contents based on HPLC analyse. Current study showed that *A. anthlloides* (208,95±4,09 µg/g) and *A. hirsutus* (200,9±2,8 µg/g) have high γ-tocopherol content. Also, D3 vitamin content of studied *Astragalus* species were found between 2,94±0,18 µg/g (*Astragalus odoratus*) and 12,53±,495 µg/g (*Astragalus anthlloides*). On the other hand, α-tocopherol content of *Astragalus* species have detected between 3,99±0,14 µg/g (*Astragalus campylorhynchus*) and 11,9±0,66 µg/g (*Astragalus cephalotes* var. *cephalotes*). However, it was found that *A. hirsutus* only have beta caroten content (3,55±0,43 µg/g) among studied species. On the other hand, it was found that r-tocopherol, a-tocopherol acetate, D2, K1, retinol and retinol acetate contents were lowest amounts or absent.

Keywords: *Astragalus*, HPLC, Lipide-Soluble Vitamins.

Bazı *Astragalus* Türlerinin HPLC Kullanılarak Yağda Çözünen Vitamin İçeriklerinin Belirlenmesi

Öz

Bu çalışmanın amacı, HPLC analizlerine göre lipitte çözünen vitamin içeriklerini belirleyerek beş *Astragalus* türünün (*Astragalus anthlloides*, *Astragalus hirsutus*, *Astragalus campylorhynchus*, *Astragalus cephalotes* var. *cephalotes*, *Astragalus odoratus*) biyokimyasal çalışmalarına katkıda bulunmaktır. Bu çalışma, *A. anthlloides* (208,95±4,09 µg/g) ve *A. hirsutus* (200,9±2,8 µg/g)'un yüksek γ-tocopherol içeriğine sahip olduğunu göstermiştir. Ayrıca, çalışılan *Astragalus* türlerinin D3 vitamin içerikleri 2,94±0,18 µg/g (*Astragalus odoratus*) ve 12,53±,495 µg/g (*Astragalus anthlloides*) arasında bulunmuştur. Öte yandan, *Astragalus* türlerinin α-tokoferol içerikleri 3,99±0,14 µg/g (*Astragalus campylorhynchus*) ile 11,9±0,66 µg/g (*Astragalus cephalotes* var. *cephalotes*) arasında belirlenmiştir. Bununla beraber, çalışılan türler arasında sadece *Astragalus hirsutus*'un beta karoten içeriğine (3,55±0,43 µg/g) sahip olduğu bulunmuştur. Ayrıca, r-tokoferol, a-tokoferol asetat, D2, K1, retinol ve retinol asetat içeriklerinin ya çok düşük oranlarda olduğu ya da hiç bulunmadığı bulunmuştur.

Anahtar kelimeler: *Astragalus*, HPLC, Yağda Çözünen Vitaminler.

1. Introduction

Astragalus L. is one the of biggest members of *Leguminosae* which comprises about three thousand species in the world [1-3]. Species of *Astragalus* is distributed generally around in the temperature and arid areas of the world including Northern and Southern America, Asia and Europe [4-6]. Many members of genus are herbaceous, annual, non-climbing plants [7]. Most species of genera are narrow

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endemics [3]. In Turkey, the genus includes four hundred taxa in 62 sections [1,8,9]. It is generally spread in the steppe areas in the of mountains in Turkey [2].

Astragalus is utilized traditionally as medicinal herbs since old ages [10-12]. It is used usually as herbal supplement, herbal tea and mixed with different constituents to heal the chronic bronchitis, hypertension, cough and ulcer [10,12,13]. Various studies showed that compounds of *Astragalus* have antitumor, antioxidative, antidiabetic, immune system modulatory, hepatoprotective, neuroprotective, regulations of cardiovascular, antiviral and antitumor effects [14-18]. Also, they possess therapy of adrenal glands, lung disorders and gastrointestinal system [11,19]. Several biochemical studies demonstrated that major components of *Astragalus* are polysaccharides, amino acids, vitamins, phenols, amino acids, saponins, alkaloids, sterols [20-22]. Studies about species of *Astragalus* generally are about polysaccharides, phenolics and terpenoids. It was seen that there was not enough study about lipide-soluble vitamin contents of *Astragalus* when reviewing literature. Therefore, the target of this study is specified to contribute biochemical studies of *Astragalus* by determining the lipide-soluble vitamin contents.

2. Materials and Methods

2.1. Plant Materials

Lipide-soluble vitamins compositions in seeds of the *Astragalus* L. taxa (*Astragalus anthlloides* Lam., *Astragalus leporinus* var. *hirsutus*, *Astragalus campylorhynchus*, *Astragalus cephalotes* Banks. & Podlech var. *cephalote*, *Astragalus odoratus* Lam.) were examined in this study (Table 2).

2.2.Extraction of Plant Materials

1 g seed was homogenised in solvent isopropanol/hexane/ (2:3 v/v) [23] and was treated at 10.000 g along five minutes. Afterwards, at 40°C, extracts were treated on a rotary evaporator. Then, samples were prepared based on the method of Sánchez-Machado [24].

Table 1. Localities of studied *Astragalus* taxa

Taxa	Locality
<i>A. anthlloides</i> Lam.	Elazığ, Baskil, Hasan Mountain, Elazığ, 1850 m. 2007.
<i>A. leporinus</i> var. <i>hirsutus</i>	Elazığ, Baskil, Bekci mezarası, Yamaclar, 1500-1750 m. 2007.
<i>A. campylorhynchus</i>	Elazığ, Baskil, 1410-1480 m area surrounding Bolucuk district, 2007.
<i>A. cephalotes</i> Banks. & Podlech var. <i>cephalotes</i>	Elazığ, Baskil, Kayabeyli village 1430 m, 2007.
<i>A. odoratus</i> Lam.	Elazığ, Baskil, Hasan Mountain, 1900-2000 m. 2007.

2.3. HPLC analysis of vitamins

All of analysis were conducted by HPLC. Seeds were dissolved in mobile phase (methanol/acetonitrile; 25/75 v/v) and were injected 50 µL. The temperature of analytical column was performed at 40 °C. Detection of retinol acetate and retinol were done at 320 nm, and the detection of D2, D3, α-tocopherol acetate, α-tocopherol, δ-tocopherol were done 215 nm for, detection of K1 was done 235 nm. Authentic external standard mixtures were used to detect the vitamins [25]. The findings obtained from analysis were represented as µg/g.

3. Results and Discussion

The detailed results of lipide-soluble vitamins in studied *Astragalus* taxa were given Table 2.

Table 2. The lipide-soluble vitamin contents of five *Astragalus* taxa

Species	Lipide-soluble vitamins (µg/g)										
	Beta-carotene	Gamma tocopherol	R-tocopherol	D2	D3	a-tocopherol	a-tocopherol acetate	K1	Retinol	Retinol acetate	
<i>A. anthilloides</i>	-	208,95±4,09	-	0,07±0,15	12,53±,495	5,67±0,26	1,26±,011	-	0,49±0,041	-	
<i>A. leporinus</i> var. <i>hirsutus</i>	3,55±0,43	-	-	-	4,48±,12	3,78±0,16	1,12±0,17	-	0,07±0,04	0,21±0,014	
<i>A. campylorhynchus</i>	-	200,9±2,8	-	-	6,44±1,12	3,99±0,14	3,41±0,27	0,07±0,02	0,3±0,02	0,07±0,018	
<i>A. cephalotes</i> var. <i>cephalotes</i>	-	-	-	-	11,48±,79	11,9±0,66	-	-	0,28±0,01	0,42±0,02	
<i>A. odoratus</i>	-	-	-	-	2,94±0,18	8,54±0,59	-	-	0,21±0,01	0,14±0,01	

In this study, it was found that *A. anthlloides* (208,95±4,09 µg/g) and *A. hirsutus* (200,9±2,8 µg/g) have high γ -tocopherol content. The other three species have not γ -tocopherol content based on present results. D3 vitamin content of studied *Astragalus* species were determined between 2,94±0,18 µg/g (*A. odoratus*) and 12,53±,495 µg/g (*A. anthlloides*). Also, α -tocopherol content of *Astragalus* species have found between 3,99±0,14 µg/g (*A. campylorhynchus*) and 11,9±0,66 µg/g (*A. cephalotes* var. *cephalotes*). However, it was found that *A. hirsutus* only have beta caroten content (3,55±0,43 µg/g) among five *Astragalus* species. Five *Astragalus* don't have r-tocopherol content in this study. The only three *Astragalus* species (*A. anthlloides*; *Astragalus leporinus* var. *hirsutus*; *A. campylorhynchus*) have a-tocopherol acetate content (1,26±,011 µg/g, 1,12±0,17 µg/g, 3,41±0,27 µg/g, respectively). On the other hand, D2, K1, retinol, retinol acetate contents of studied five *Astragalus* species found trace amounts or absent.

4. Conclusions

Legumes are the most significant foods consumed by humans especially in many areas of world including Asia, Europe and Africa [26]. Several studies indicated that legumes have complex carbohydrates, vitamins, fibers, polyphenols [27]. Results from present study demonstrated that studied five *Astragalus* species have D3 vitamin content of studied *Astragalus* species were found between 2,94±0,18 µg/g and 12,53±,495 µg/g. Whereas, these study showed that *Astragalus leporinus* var. *hirsutus* have 3,55±0,43 µg/g beta-carotene content. However, E-Siong et al. [28] indicated that for most of the legumes, the major carotenoids detected were b-carotene, lutein and cryptoxanthin. Fernandez-Marin et al. [29] found that total carotenoids of legume seeds were between 01.±0.1 µg/g and 17.7.±2.2 µg/g. in another study done by E.-Siong et al. [28] it was found that carotene content of legumes were 9.2±10 mg/kg. Valdivielso et al. [30] indicated that beta-carotene content of legume species were 2.91 ± 0.148 mg/100g.

Tocopherols, are lipide-soluble vitamins which play active role the scavenging of free radicals in the cell [31]. α -tocopherol content of *Astragalus* species have found between 3,99±0,14 µg/g and 11,9±0,66 µg/g, int this study. A-tocopherol was found as main content study by Valdivielso et al. [30]. A-tocopherol contents of studied three species in present study were found 1,12±0,17 µg/g and 3,41±0,27 µg/g apart from *A. cephalotes* var. *cephalotes* and *Astragalus odoratus* which don't have a-tocopherol content. Data obtained from present work determined that *A. anthlloides* (208,95±4,09 µg/g) and *Astragalus leporinus* var. *hirsutus* (200,9±2,8 µg/g) have high gamma tocopherol content among studied five taxa. Smilarly, Wyatt et al. [32] found that γ -tocopherol was high levels. Also, Cho et al. [33] found that legumes were found to contain only γ -tocopherols (86.1–146.8 mg/kg). Fernandez-Marin et al. [29] determined that γ -tocopherol was the most abundant isoform in all legume species, except for *Vigna* and *Arachis*, where δ -tocopherol and α -tocopherol were the main isoforms, respectively. Present study indicated that studied r-tocopherol, D2, K1, retinol and retinol acetate contents of *Astragalus* species were lowest amounts or absent.

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