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The lipid-soluble vitamins contents of some *Vicia* L. species by using HPLC

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Abstract: In the present study, lipid-soluble vitamin compositions in the seeds of the *Vicia* L. taxa (*V. ervilia* (L.) Willd., *V. cuspidata* Boiss., *V. peregrina* L., *V. cracca* L. subsp. *stenophylla* Gaudin, *V. mollis* Boiss.& Hausskn., *V. hybrida* L., *V. sativa* L. subsp. *nigra* (L.) Ehrh. var. *nigra* L., *V. sativa* L. subsp. *sativa* (Ser.) Gaudin var. *sativa, V. crocea* (Desf.) B. Fedstch., *V. noeona* Reuter ex Boiss. var. *noeona, V. narbonensis* L. var. *narbonensis*) were determined by using HPLC. It was found that studied *Vicia* species apart from *V. ervilia* and *V. cuspidata* have highest ß-carotene contents, 1523,7±6,4 µg/g and 236,62±1,8 µg/g, respectively. Also, this study showed that *V. ervilia* and *V. cuspidata* have highest γ-tocopherol content. On the other hand, current study indicated that *Vicia* species have D3 vitamin contents between 13,8±0,62 µg/g and 50,5±2,13 µg/g. However, the α-tocopherol, α-tocopherol acetate, D2, K1, retinol and retinol acetate contents of studied *Vicia* L. species were lowest.

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1. INTRODUCTION

Fabaceae, is the third major family of higher plants, contains about 730 genera and 19400 species and are second to Poaceae in agricultural and economic priority [1-4]. The members of family are harvested as yield and used in many areas such as medicines, fuel and chemistry [5]. They are oldest crops consumed as important source of protein in the various areas of world including Europe, Middle East, Africa, and South Asia [6-8]. These are rich in carbohydrates, proteins, minerals, polyunsaturated fatty acids, fibers, carotenoids and vitamins [9-11].

Vicia L., is located tribe *Viciaeae* of the Papilionoideae, is includes about 210 species and divided into two subgenera, Vicia and Vicilla (Schur) Rouy and also 22 sections [12-14]. The species of *Vicia* are widely distributed Europe, Asia and America [15]. It was suggested that

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the Mediterranean region is the main centre of diversification of *Vicia* and is represented by 64 species, 22 subspecies and 18 varieties in Turkey [16-21]. *Vicia* L. is most significant sources for human diet in the many regions of world [22-25].

Not only plants consumed as food they can also used as the therapeutic effects against different diseases [26,27]. Although some studies performed about determining phenolics, proteins and fatty acid compositions of legumes including *Vicia* there were no enough information about the lipid-soluble vitamins contents [28-31]. Vitamins are important both human metabolism and also redox reactions in the plants [32]. Lipid-soluble vitamins including tocopherols and carotenoids, are non-enzymatic components of antioxidant system, have important role to protect cell membranes against free radical damage by working together [33-35]. Vitamin E, is a most powerful interrupting chain breaking antioxidant, defends cell membrane fatty acids agaist lipid peroxidation [36]. Also, carotenoids supply antioxidative preservation against lipid rich tissues [37-38]. It has been demonstrated that there is an inverse linking between the dietary intake of antioxidant-rich food and apparance of human diseases [39]. Studies showed that α-tocopherol, γ-tocopherol, ß-carotene, lycopene and lutein inhibit cancer, coronary heart disease, neurological diseases, diabetes [40].

The major goal of present study is to determine the lipid-soluble vitamin contents of some *Vicia* L. taxa. Hereby, it was intended to contribute the biochemical studies of legumes which not enough study about vitamin contents. It is thought that such biochemical studies will provide support to researchers working legumes and also antioxidants.

2. MATERIAL and METHODS

2.1. Collection of Plant Materials

Sample plants were gathered from the natural habitats and were stored in the Firat University Herbarium (FUH;4001-4011). The species were identified by Dr. Ahmet Şahin and Dr. Hasan Genç. Details about the materials are explained in Table 1.

Table 1. Localities of studied *Vicia* L. taxa

Taxa	Section	Locality
Vicia ervilia	Ervum	Elazig, Fırat University campus, 1060 m
Vicia cuspidata	Vicia	Elazig –Sivrice, Firat University Camp, 1100 m
Vicia peregrina	Vicia	Elazig, Fırat University campus, 1060 m
Vicia mollis	Vicia	Elazig, Hankendi Tilek hill, 1050 m
Vicia hybrida	Vicia	Malatya-Poturge, Gunduzkoy, 1250 m.
Vicia sativa subsp. *nigra* var. *nigra*	Vicia	Elazig, Fırat University campus, 1060 m
Vicia sativa subsp. *sativa* var. *sativa*	Vicia	Elazig, Fırat University campus, 1060 m
Vicia noeona var. *noeona*	Vicia	Elazig-around Cip dam, 1100 m
Vicia crocea	Cracca	Trabzon-Araklı, 1900 m
Vicia cracca subsp. *stenophylla*	Cracca	Elazig, Degirmenonu village, 1100 m
Vicia narbonensis var. *narbonensis*	Faba	Elazig-Sivrice, Firat University camp, 1100 m.

2.2.Extraction of plant materials

1 g seed used to analyse the lipid-soluble vitamins contents. The seeds are homogenised with hexane/isopropanol (3:2 v/v) [41]. The solvent was removed on a rotary evaporator at 40°C after samples were centrifuged at 10.000 rpm for 5 minutes. After that lipid-soluble vitamins were extracted according to the method of Sánchez-Machado [42]. Laboratory works were repeated three times.

2.3. Chromatographic analysis and quantification of lipid-soluble vitamins

Samples were vortexed and kept at 85 ºC for 15 min. After 5% KOH added to samples to determine the vitamin contents. Following, the tubes were cooled to room temperature and pure water was added and stirred. The non-soaped lipophilic molecules were extracted with 2x5 ml hexane. Later, hexane phase was evaporated using nitrogen gas. Finally, residue was solved 1 ml methanol/acetonitrile (25/75 v/v) and taken to autosampler tubes.

The column used was a HPLC Column A Supelcosil ^{TM} LC18 column (250 x 4.6 mm, 5 mm, Sigma, USA). The elution was performed at the flow rate of 1 ml/min. The analytical column was worked at 40 °C. One ml extract gained by saponification and it is subtilized by using 500 µl of the mobile phase. It was used LC-10 ADVP UV-visible as pump, SPD-10AVP as detector, CTO-10ASVP as column furnace, SIL-10ADVP as otosampler, DGU-14A as degasser unite. Detection was conducted at 320 nm for retinol acetate, retinol, 215 nm for vitamin D2, D3, γ-tocopherol, α-tocopherol acetate, α-tocopherol, 235 nm for vitamin K1 and 450 nm for ß-carotene. Identification of the vitamins were performed by external standard mixture. Class Vp 6.1 software assisted at workup of the data [43]. The results of analysis were uttered as µg/g for samples.

3. RESULTS

The lipid-soluble vitamins contents of *Vicia* L. taxa were given in Table 2. It was found that *V. peregrina* has highest ß-carotene content (1523,7±6,4 µg/g) in this study (Table 2). *V. sativa* subsp. *nigra* var. *nigra* (614,6±4,5 µg/g) and *V. sativa* subsp. *sativa* var. *sativa* (746,2±3,3 µg/g) have high ß-carotene content among studied 11 *Vicia* species. The other species have between 236,62±1,8 µg/g and 452,8±3,5 µg/g except for *V. ervilia* and *V. cuspidata.* However, *V. ervilia* and *V. cuspidata* have quit high the γ-tocopherol content (1326±3,21 µg/g; 2148±4,12 µg/g, respectively). Also, *V. narbonensis* have 86,3±2,11 µg/g γ-tocopherol content. The other studied species have low amounts or don't have γ-tocopherol content. It was found that r-tocopherol and D2 contents were quit poor. The D3 vitamin contents of studied *Vicia* species found between 50,5±2,13 µg/g (*V. naeona* var. *naeona*) and 13,8±0,62 µg/g (*V. hybrida*). It was found that *Vicia mollis* have high α-tocopherol content (14,3±1,19 µg/g) while *V. cuspidata* have low amount (0,7±0,1 µg/g). *V. ervilia* don't have α-tocopherol acetate content. *V. cracca* subsp. *stenophylla* (8,8±0,9 µg/g), *V. cuspidata* (5,7±1,1 µg/g), *V. mollis* (2,5±0,2 µg/g), *V. sativa* subsp. *sativa* var. *sativa* (2,2±0,2 µg/g) and *V. peregrina* (0,3±0,01 µg/g) have K1 vitamin content among studied *Vicia* species. The other studied lipid-soluble vitamins (α-tocopherol, α-tocopherol acetate, D2, K1, retinol, retinol acetate) were low amounts or absent (Table 2).

Table 2. The lipid-soluble vitamins contents of studied *Vicia* L. taxa

	Lipid-soluble vitamins (µg/g)									
Taxa	ß-carotene	γ tocopherol	R-tocopherol	D2	D3	α -tocopherol	α -tocopherol acetate	K1	Retinol	Retinol acetate
Vicia ervilia	-	1326±3,21	-	-	18,8±,0,97	-	-	-	0,2±0,02	0,9±0,06
Vicia cuspidata	-	2148±4,12	-	-	33,9±1,49	0,3±0,03	0,7±0,1	5,7±1,1	0,2±0,03	0,9±0,04
Vicia peregrina	1523,7±6,4	-	-	-	34,4±1,49	0,8±0,02	2,2±0,4	0,3±0,01	0,4±0,01	0,6±0,03
Vicia mollis	331,6±3,1	-	-	-	18,2±0,95	-	14,3±1,19	2,5±0,2	0,1±0,01	0,2±0,01
Vicia hybrida	452,8±3,5	-	-	-	13,8±0,62	0,3±0,01	5,4±1,1	-	0,1±0,01	0,2±0,03
Vicia sativa subsp. *nigra* var. *nigra*	614,6 ±4,5	-	-	-	33,8±1,12	0,2±0,04	1,7±0,3	-	0,3±0,01	0,7±0,02
Vicia sativa subsp. *sativa* var. *sativa*	746,2±3,3	7,9±0,98	-	-	32,9±0,94	1,1±0,1	1,5±0,4	2,2±0,2	0,2±0,02	0,4±0,03
Vicia naeona var. *naeona*	350,94±2,7	-	-	0,9±	50,5±2,13	0,1±0,01	2,2±0,1	-	0,4±0,02	0,7±0,05
Vicia crocea	439,4±1,6	0,6±0,04	-	-	12,5±0,85	1,9±0,7	7,1±0,12	-	-	0,2±0,01
Vicia cracca subsp. *stenophylla*	347,8±3,8	-	-	-	14,1±0,94	0,6±0,01	2,9±0,6	8,8±0,9	-	0,2±0,01
Vicia narbonensis var. *narbonensis*	236,62±1,8	86,3±2,11	0,3±0,01	-	27,6±1,31	0,6±0,03	3,1±0,1	-	0,2±0,01	0,5±0,03

4. DISCUSSION

Legumes are low in calories and significant source of protein, vitamin, starch, mineral, fiber and pyhonutrient content [11,44]. They have protective role against various diseases such as cardiovascular, cancer and diabet [45,46]. Present study showed that studied *Vicia* species apart from *V. ervilia* and *V. cuspidata* have highest ß-caroten content between 1523,7±6,4 µg/g and 236,62±1,8 µg/g. Carotenoids including ß-carotene and α-carotene have important role due to provitamin A activity [47]. They play vital role the protection against cancer, cardiovascular diseases [48-49]. However, Mamatha et al. [47] found that studied legumes including *Phaseolus, Vigna, Lens* and *Cicer* have lowest α-and β-carotene contents. Similarly, Sahin et al. [50] found that *Vicia* species have lowest β-carotene content between 0,96 µg/g and 0,3 µg/g. And also it was found that legumes don't have β-carotene content a study done by Yao et al [51].

Vitamin E, the other studied lipid-soluble vitamins, which has several health benefits such as hypoglisemic, antihypertensive, neuroprotective and anti-inflammatory [52-54]. In this study, it was found that some species from *Vicia* (*V. ervilia* and *V. cuspidata;* 1326±3,21 µg/g; 2148±4,12 µg/g, respectively) have high the γ-tocopherol content while the other species have low amounts or don't have γ-tocopherol content except for *V. narbonensis*. However, Bağcı et al. [45] indicated that α and γ–tocopherol were detected as the most abundant tocopherol components in all of the studied taxa except *Lathyrus inconspicuus, Onobrychis luetiana* and *Onobrychis hypargyrea.* It was determined that α-tocopherol acetate was the major compound in all the botanical species except for *R. bulbosus* in which free α-tocopherol was the major compound work done by Valdivielsa et al. [56]. Present study determined that *Vicia* species have D3 vitamin contents (13,8±0,62 µg/g-50,5±2,13 µg/g). It was showed that *Lathyrus*, the other genus from legume, has vitamin D3 content between 8,27±0,04 µg/g and 45,07±2,23 µg/g (Sahin et al., 2009). On the other hand, the other studied lipide-soluble vitamins (α-tocopherol, α-tocopherol acetate, D2, K1, retinol, retinol acetate) were low amounts or absent based on present results. Similarly, Sahin et al. [57] found that retinol acetate and vitamin D2 contents of *Lathyrus* low or absent. Another study showed that *Vicia* species have vitamin A content between 0,397 µg/g and 0,922 µg/g [57]. Also, it was determined that *Vicia* species have high vitamin E content (2,778-19,19 µg/g) study done by Sahin et al. [50]. However, Sahin et al. [50] indicated that *Lathyrus* has vitamin K1 content between 2,1±0,06 µg/g and 11,15±0,08 µg/g. Also, they found that *Lathyrus* has α-tocopherol content between 11,51±0,2 µg/g and 67,22±0,14 µg/g.

5. CONCLUSION

In conclusion, this study presented that studied *Vicia* taxa have highest β-carotene contents except for *V. ervilia* and *V. cuspidata* which have highest γ-tocopherol content. Also, studied *Vicia* taxa have high D3 vitamins. It was fouınd that the other studied lipid-soluble vitamins low amounts or absent in this study.

Conflicts of Interest

Authors declare no conflict of interest.

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