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Flowers, Pollen and Honey for Use in the Treatment of Parkinson's Disease

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Abstract: *Honey is nectar collected from plants and produced by honeybees Apis mellifera. It has variable sensory and bioochemical properties due to climate and environmental conditions as well as the different origins of the plants in which it is harvested. Studies have shown that the antioxidant potential of honey varies greatly with the source of flowers. Vicia faba L. also known in Turkey as "bakla," is a genus of the family Fabaceae that is widely grown in Asia. It is abundant in L-DOPA, a therapeutic agent used in Parkinson's disease diagnosis. In this study, a sample of honey has been obtained from a monofloral source in the greenhouse environment where only vicia faba l. was found. The biochemical properties and antioxidant activities of this honey sample were studied. Vicia faba L. flowers grown in greenhouse conditions and pollen and honey produced by bees fed from this flowers were analyzed with HPLC method. The results were quite surprising. L-DOPA content is 4.23% in flowers, 0.98% in pollen and 0.076% in honey. This leads to the conclusion that Parkinson's patients can continue treatment by using only pollen and honey produced by bees that feed on Vicia faba L. flowers. In addition, antioxidant activity of flowers, pollen and honey was analyzed by spectrophotometric methods. Parallel results were obtained with the results previously studies.*

Keywords*: Vicia faba, Flowers, L-DOPA, Honey, Pollen*

1. Introduction

Faba beans (*Vicia faba* L.) is one of the important plants of the Mediterranean cuisine which dates back to prehistoric regions in Mesopotamia and Europe [1]. Faba bean is an important plant that can fix symbiotic nitrogen to the soil, make soil nitrogen usable and increase and maintain soil fertility, in addition to its use as food and feed [2]. With its seeds rich in protein and energy and its ability to grow in various climatic regions, faba bean production has many different uses as feed and food [3].

Faba bean seeds are rich in protein, carbohydrates, minerals, fiber and B group vitamins. Faba bean is one of the rare plants that contain L-DOPA (L-3,4-dihydroxyphenylalanine) in their leaves, flowers and fruit parts that can be used in the treatment of Parkinson's disease which is common in older societies [4]. Parkinson's disease (PD) is a common neurodegenerative disease characterized by loss of muscle control, which causes tremors of the limbs and disruption of the head balance. [5] have reported that several plant species have the potential to be developed as a functional food product for PD patients due to the L-DOPA content of faba beans.

Faba beans have also been reported to be a rich source of many bioactive nourishing compounds, including phenolic antioxidants [6]. The most common herbal phenolic antioxidants are flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids [7]. These substances are highly reactive molecules with reactive oxygen species and are produced as a result of normal metabolism in cell organelles, especially in the mitochondria, or due to causes such as reperfusion, aging, radiation, high oxygen pressure, inflammation and exposure to chemical agents [8]. Defense systems that work in

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the body to prevent the formation of reactive oxygen species, prevent damage caused by these substances and provide detoxification are called “antioxidant defense systems” or “antioxidants” [9]. It was shown in various studies that faba beans are a good source of antioxidants [10, 11].

Faba beans also contain several anti-nutritional compounds such as vicine, convicine, and tannins in their seed, which negatively affect their digestibility [12]. Vicine and convicine, which are pyrimidine glycosides, accumulate in cotyledons [13]. G6PD (Glucose-6-Phosphate Dehydrogenase) deficiency is one of the most common hereditary diseases and shows hereditary transition due to the X gene. G_6PD enzyme is found in all tissues. In the deficiency of this enzyme that catalyzes the first step of the Pentose Phosphate Pathway (PFP), neonatal jaundice or acute hemolytic anemia may develop after infection, use of some medications, or ingestion of faba beans [14]. Faba bean is the only plant that causes hemolytic anemia in individuals with G_6PD enzyme deficiency, and the course of hemolytic anemia caused by this plant is called favism (15]. Approximately 400 million people are affected by this health disorder (16].

Faba beans have a high rate of foreign pollination due to the reasons such as the fact that the pollen sacs remain under the stigma and, in general, pollen does not fall on the stigma. Also, it has the ability to creating an unlimited number of flowers. However, depending on the characteristics of flower organs, some negative factors after fertilization and environmental conditions, faba bean can turn only 12-33% of the many flowers it produces into fruit [17].

Faba bean plant is a cool climate legume and blooms in early spring when many plants do not bloom and it can be a source of pollen and nectar for many insects. In the pollinated plants, insects belonging to the Apoidee family, which includes the bees in the pollination rates, are active. Faba bean plant blossoms their flower petals during the flowering period and attracts the bees because they have the strength and weight to reach male organs and nectar [18]. Insufficient pollination causes significant reductions in pod yields. In a study in which pollination with honey bees was experimented, as a result of bee pollination, the number of pods, the number of seed per pod and yield increased, while the ripening time decreased [19). Insects such as bees, etc. that provide foreign pollination in different studies have been reported to increase yields by 40 to 185% in faba bean [20].

Honey produced by honey bees (*Apis mellifera*) from nectar and plant extracts, is a natural product containing a complex carbohydrate mixture and a small number of other ingredients, mainly minerals, proteins, vitamins, organic acids, phenolic compounds, enzymes and other phytochemicals [21]. The phenolic composition in honey mainly depends on the origin of the flower. In general, the flavonoids found in honey are flavones, flavanols, and flavonols [22]. Phenolic Acids are known as 2-cis,4-trans Abscisic acid, 2-Hydroxycinnamic acid, Caffeic acid, Chlorogenic acid, Cinnamic acid, Ellagic acid, Ferulic acid, Gallic acid, p-Coumaric acid, p-Hydroxybenzoic acid, Protocatechuic acid, Sinapic acid, Syringic acid, and Vanillic acid [23].

L-DOPA is the main ingredient of many prescribed drugs used in the treatment of PH. Topal and Bozoğlu [4], in their study in which they investigated L-DOPA contents in faba beans, flowers, and fruits, found that the highest L-DOPA content was in the flowers. Therefore, in a study where the L-DOPA content was examined in the flowers of the faba beans and the tea samples produced from these flowers, L-DOPA value in dried flowers was 6.85-8.38 g/100 g while it was 7.40-8.60 g / 100 g in the tea made by brewing dried flower samples in boiling water for 15 min [24].

Due to the unlimited growth characteristics of a faba bean plant, it blooms a lot, but most of the blooming flowers do not set pods and yield grains, either the flowers and pods are shed a significant loss of energy occurs. Also, faba beans are one of the earliest flowering plants in spring due to their low total temperature demand. To increase the pollination and fertilization, faba bean needs bees, and the bee needs faba beans because it blooms when other plants do not bloom. The bees increase the number of pollinated flowers and therefore the rate of pod setting, change the content of their honey and pollen due to the plant they visit. This study was carried out with the hypothesis that L-DOPA, which is found in brad bean flowers, can pass to honey and pollen of bees fed with these flowers and thus such a result will pave the way for future studies on the use of these products in healthy nutrition. The aim was to detect some secondary metabolites such as L-DOPA and vicine in honey and pollen obtained from faba

beans grown under controlled greenhouse conditions. Especially in bean honey and bee pollen, these contents were examined for the first time. The ingredients in the flower, honey and pollen samples obtained from the faba beans were determined to make a new approach by drawing attention to the use of faba bean plants as medical purposes as well as food. One of the ultimate goals in the present study was to expand the use of faba bean.

2. Materials and methods

2.1. Experimental Conditions

The study was carried out under the conditions of Bilecik Şeyh Edebali University Faculty of Agriculture and Natural Sciences Greenhouse (450 m^2). The Sevil faba bean variety, an early variety, was used in the study. Faba bean sowing was carried out at 50x15 cm intervals and, to prolong the flowering period, on two different dates: February 15 and March 14, 2019. After flowering, a small (about 800 apiary) beehive was placed in the greenhouse on April 22, 2019. Anatolian Honey Bee (*Apis mellifera anatoliaca*) was used as the bee race. The greenhouse area was taken under control with thin mesh-like plastic material to prevent the exit of bees. The flower sample was taken on the date of the installation of the hive and it was kept at +4 ^{0}C until the time of analysis. Pollen and honey samples were taken from the hive towards the end of June and stored at +4 ^{0}C until the time of analysis.

2.2. Sample Extraction

The extraction procedure was performed in an ultrasonic bath (Bandelin Sonorex with a frequency of 50 kHz) to determine the total phenolic, flavonoid and antioxidant capacity. Accordingly, a 1 g sample was weighed and extracted with 30 mL 70% methanol solution for 30 min. After the extraction, the mixture was filtered using a Whatman white filter paper and stored in the refrigerator at + 4°C until the time of further analysis.

2.3. Analysis of Total Phenolics

Total phenol content (TPC) was determined using the Folin-Ciocalteu test [25]. A 0.2 mL sample of extract and 0.5 mL Folin-Ciocalteu reagent (diluted 10 times with water) were added to the test tubes. The solution was then kept in the dark for 5 min and then 1 mL of sodium carbonate (7.5% w/v) was added. The tubes were covered with parafilm and kept in the dark again for one hour. The absorbance at 765 nm was measured by a UV-Vis spectrophotometer (Shimadzu UV 1800). The results were compared to the gallic acid calibration curve. Results are expressed as mg gallic acid/g dry sample. Each experiment was run in three replicates.

2.4. Total Flavonoid Analysis

The total flavonoid content of the raw extract was determined by the aluminum chloride colorimetric method [26]. The absorbance values of gallic acid standard solutions in five different concentrations were measured with Shimadzu UV-1800 spectrophotometer at 765 nm wavelength.

2.5. Antioxidant Activity Analysis (DPPH)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) test was adopted by modifying the method developed by Brand-Williams et al. [27]. A 24 mg DPPH stock solution was weighed and dissolved in some methanol. Then, it was transferred to a 100 mL flask, and the volume was completed to 100 mL with methanol It was then stored at -18°C until use. The analysis solution was obtained by mixing 90 mL of methanol with 20 mL of stock solution to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using a spectrophotometer.

A 300 µL extract sample was taken in a tube and 5.700 µL of DPPH solution was added. The mixture was allowed to react for one hour in a dark place. Then the absorbance value of the solution was measured at 515 nm wavelength in the spectrophotometer. Antioxidant activity was calculated as a decrease in absorbance value using the formula:

$$\text{Antioxidant activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Accordingly,

A0: The absorbance value of the sample-free control.

A1: The absorbance of the mixture containing the sample.

The absorbance results were converted using the calibration curve of the ascorbic acid standard and expressed as the equivalent of ascorbic acid.

2.6.L-DOPA and Vicine Analysis

2.6.1.Sample preparation

For L-DOPA and vicine analysis, since they are well-soluble chemical agents in water, 10 g sample for honey and pollen and 1 g sample for faba bean flower were weighed and 50 mL boiled and deionized water was added and the mixture was stirred in the ultrasonic bath for 15 min. Extracts filtered with white Whatman filter paper were stored in the refrigerator at 4^0C until the time of analysis.

2.6.2.Content analyses

Analyzes were performed using an Agilent 1260 model UV-Vis Detector HPLC device. The separation was carried out using an ACE brand C18 column (150 mm x 4.6 mm, 5 microns). The mobile phase was formed by adding 20 mL methanol and 1 mL glacial acetic acid to 979 mL deionized water. The flow was set to 1 $mL.min^{-1}$, column thermostat temperature to 30°C, detection to 283 nm, injection to 20 microliters and analysis time was set to 10 min in isocratic conditions [28].

3. Results and discussions

3.1.Total Phenolics

Total phenolics are a heterogeneous class of chemical compounds formed by polyphenols, flavonoids, and phenolic acids. Polyphenols are generally the product of secondary plant metabolism and are characterized by the presence of multiple phenolic groups associated with more or less complex structures [23]. Polyphenols are considered to be the main ingredients responsible for the antioxidant activity of honey, associated with the ability of free radical scavengers. Phenolic compounds stabilize free radicals when they give hydrogen from one of their hydroxyl groups, their degree of activity is related to the number of hydroxyl groups [29]. In the present study, the standard curve of the measurements is given in Figure 1 and the total phenolic contents are given in Figure 2.

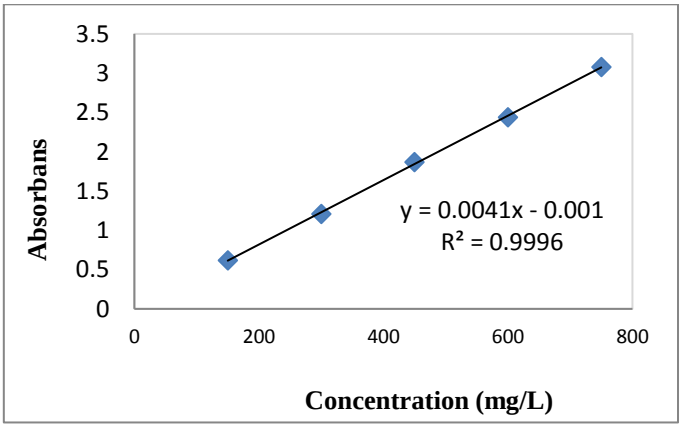

Figure 1. The gallic acid standard curve (TFC)

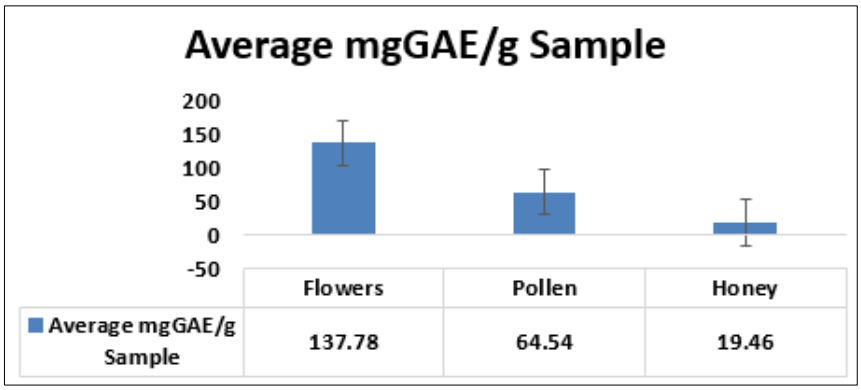

Figure 2. Total phenolic contents of faba bean flowers, pollen, and honey

Since there is no evidence of a direct link between the phenolic contents and faba bean (*Vicia faba L.*) flower, pollen and honey in the literature, the results were compared with the studies conducted in other plants.

It has been reported that bee pollen is rich in phenolic compounds and it acts as a free radical scavenger and lipid peroxidation inhibitor originating from flavonoids it contains and it has antioxidant properties [30]. Duarte et al. in their study to determine the physicochemical and antioxidant profiles of honey and pollen produced by honey bees (*Apis mellifera scutellata*) in Brazil, the highest total phenolics value in honey collected from natural areas was 1.36 mgGAEq/g and 21 mg in pollen[31]. In another study, the total phenolic content in honey has been reported as 15.84 mg/100 g [32]. Al-Mamary et al have reported that the total phenolic contents ranged from 56.32 to 246.21 mg/100 g in their study on five types of Yemen honey and four types of imported honey [33].

Aker, in six different types and a total of 65 jar honey samples collected from different regions in Turkey (Yayla honey from Erzurum and Sivas, pine honey from Muğla, chestnut honey from Sinop, acacia honey from Trabzon, citrus honey from Antalya and sunflower honey from Samsun), found that the highest flavonoid level, phenol level, and antioxidant activity was in flower, honey. The highest total phenolic (mgGAE / 100g honey) content determined in honey samples was in pine honey (166.46 ± 5.80) whereas the lowest value was found in flower honey (106.04 ± 9.55). As seen in Figure 2, the total phenolic content was found to be 19.46 mgGAEq / g in faba bean honey and 64.52 mgGAEq/g in pollen. It was thought that the differences were related to floral differences. According to the results, since flavonoid and phenolic substances are of vegetable origin, it can be argued that 60.9% of these substances which the bee takes from the faba bean pass to the product [34].

3.2 Total Flavonoid

Absorbances for catechin standard solutions in five different concentrations were measured at 510 nm using a Shimadzu UV-1800 spectrophotometer. The standard curve of the measurements is given in Figure 3 and the total Flavonoid contents are given in Figure 4.

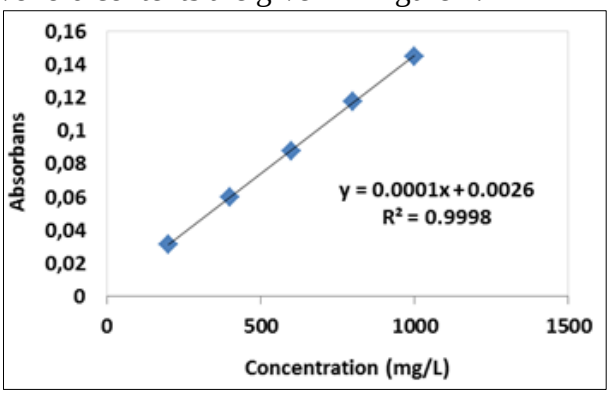

Figure 3. Catechine standard curve (TFC)

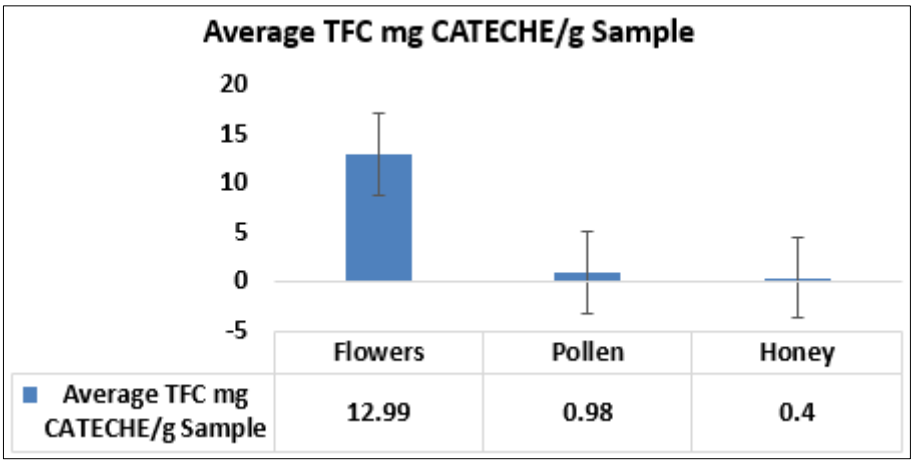

Figure 4. Total flavonoid contents of faba beans, pollen, and honey

Fadzilah et al investigated the polyphenol, total flavonoid content and antioxidant capacities of bee pollen. The researchers determined that pollen possesses high antioxidant activity due to the presence of polyphenols and flavonoids, and they determined the TPC value of pollen as 135.93 mg GAE / g and TFC value as 3180 mg QE / g [35]. The antioxidant content, on the other hand, was found to be 0.86 ± 0.01 mg/mL when pollen gave a higher rate of DPPH inhibition with EC50. Examining Figure 2, the total phenolic content of pollen was found to be 64.54 mg GAE / g sample. Total flavonoid content was determined to be 0.98 mg CATECEQ/g. While the highest total flavonoid values were determined in the flower, the content in the pollen was higher than that in the honey. Honey is a natural substance that has been appreciated for its healing abilities since ancient times. Flavonoids and phenolic acid content play an important role in human health thanks to their high antioxidant and anti-inflammatory properties [23].

Duarte et al found the highest flavonoid content in T. clavipes honey sample with 55 mg QEq./100g [31]. Meda et al examined the flavonoid content in 27 honey samples collected from Burkino Faso and reported the flavonoid content between 0.17 and 8.35 (mg QE/100 g) [36]. Aker (2016), in six different types and a total of 65 jar honey samples, have reported that the lowest flavonoid (mgQE/100g honey) in flower and citrus honey samples (1.30 ± 0.21 and 1.59 ± 0.13, respectively) whereas the highest values were found in chestnut and pine honey samples (2.68 ± 0.35 and 2.76 ± 0.23, respectively) [34]. Tabatabaei determined the flavonoid content in a total of 81 pollen samples collected in different regions in Turkey (Central and Eastern Black Sea, Marmara, Central Anatolia, Mediterranean, Aegean, South-East Anatolia) in the range of 3.72 and 4.97 (MGQ/g) [37]. Tai et al, in their study on the antioxidant activity and chemical components of the flower in *Sophora viciifolia* which is a legume plant in tree form of which its flowers are consumed, determined the total flavonoid content as 237.2 ± 10.3 mg routine/g dry matter. It was thought that the honey and pollen produced in these studies were higher than faba bean honey since more than one plant was obtained from the natural environment [38].

3.3. Antioxidant Activity / DPPH

This process is based on monitoring the visible area with a spectrophotometer until the absorbance is constant. The results were calculated as % of the extract inhibiting the DPPH radical. The results are given in Figure 5.

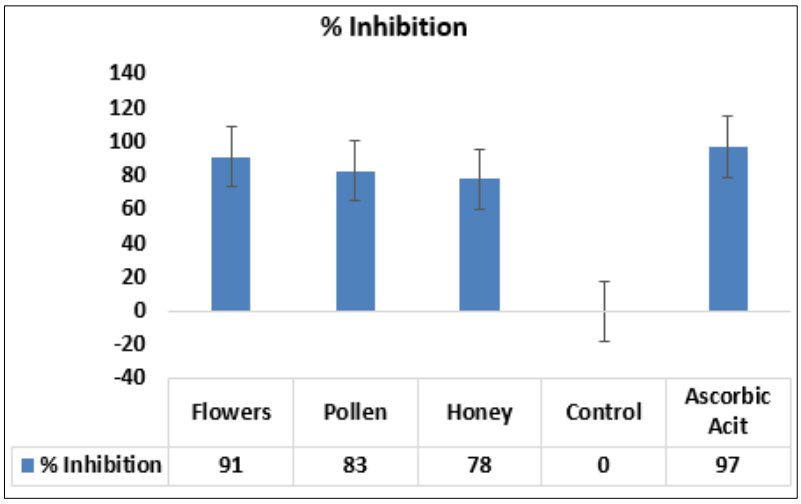

Figure 5. Total antioxidant content (inhibition %) of faba beans, pollen, and honey

Aker, in their study on honey samples in different areas in Turkey, have reported significant increases in antioxidant activity between the citrus honey and flower honey ($p < 0.005$), between chestnut honey and flower honey ($P < 0.001$), between pine honey and flower honey ($p < 0.01$) and flower honey and acacia honey ($P < 0.001$) and reported that the most effective honey was a flower honey [34].

Saral et al, in their study in which they compared the biological activities of honey bee pollen and propolis samples obtained from four bee breeds in Turkey (*Apis mellifera caucasica, Apis mellifera anatoliaca, Apis mellifera syriaca,* and *Apis mellifera carnica*), found that the flora antioxidant capacity was a more effective factor than the bee race. While total phenolic content was determined to be 58 mg GAE/100 g in honey samples, total flavonoid content was determined to be 5 mg QUEQ/100 g sample. While total phenolic content was determined to be 738 mg GAE / 100 g in pollen samples, total flavonoid content was reported as 261 mg QUEQ / 100 g sample. In the present study, the total phenolic content of honey was found to be 19.46 mg GAE/g sample. Total flavonoid content was 0.40 mg CATECEQ/g sample, total phenolic content was 64.54 mg GAE/g sample and the total flavonoid content was 0.98 mg CATECEQ/g sample. As understood from comparing the data of these two studies conducted in Turkey, similar to the results of the present study, both phenolics and flavonoid contents decrease when honey is produced from a single plant source decreases both in honey and pollen. However, this decrease was higher in pollen whereas it was lower in honey. These decreases were of no importance to us as the main objective of the present study was to determine the qualities of honey produced from faba bean plants rather than producing bee products with high content [39].

Mejías and Gloria conducted a study to determine the antioxidant activities of honey and pollen samples produced near Laima Volcano in Southern Chile as well as the metal contents and have reported the total content of phenolic substances in honey between 1000.0 and 1255.4 mg /kg, and between 347.7 in 1.286.5 mg /kg in the pollen [40].

3.4. L-DOPA and Vicine Content

The main objective of this study was to determine whether L-DOPA, which will enable the faba bean plant to be used as a medicinal plant, is transferred to bee products. In the present study, the data on L-DOPA and vicine contents determined in the flower of the Sevil faba bean variety grown under greenhouse conditions and the pollen and honey produced from the bean are given in Table 6 while the chromatograms of the standard and extracts are given in Table 1.

Table 1. L-DOPA and Vicine contents in faba bean, honey, and pollen

L-DOPA (mg/10 g)			Vicine (mg/10 g)		
Pollen	Honey	Flowers	Pollen	Honey	Flowers
4.166	0.0321	423.33	0.0853	0.0156	5.0

The highest L-DOPA (42.33 mg / g) and Vicine (5mg / g) content were determined in the flowers. The flowers of faba beans are not consumed in Turkey. In a study conducted by Topal and Bozoğlu for the detection of L-DOPA in leaf, flower and fruit samples in some faba bean genotypes, the changes in L-DOPA in genotypes were determined to be 10.88-33.41, 40.95-96.37 and 4.16-52.28 mg/kg. In the study, the highest L-DOPA content was observed in flower samples [4]. In terms of L-DOPA, one of the ways of using this valuable plant component is to produce honey from this flower, and in the present study, it was found that L-DOPA (0.0758%) in the flower passed to the honey. The amount passing to the pollen was higher (0.98%).

In the study in which the effects of factors on L-DOPA contents in faba bean were examined, Kai-Bin et al determined the average L-DOPA content in bean flowers as 4.48% [41]. In the same study, it has been reported that L-DOPA contents may vary according to flower colors, L-DOPA content decreases proportional to the darkening of the color, and the flower bud form contains more L-DOPA compared to the open flower form. In a study conducted to determine L-DOPA concentration in leaf and flower tissues in six faba bean (*Vicia faba* L.) lines with common and rare flower colors, it was found that the average L-DOPA value in dry flowers varied between 27.8 and 63.5 mg / g over dry weight [5]. In the present study, the L-DOPA content of faba bean flowers was determined to be 4.23%.

Vicine and convicine, inactive precursors of divicine and isouramil are redox compounds potentially toxic to human carriers of a widespread genetic deficiency of the erythrocyte (red blood cell, RBC) enzyme glucose-6-phosphate dehydrogenase (G_6PD). Ingestion of faba beans by these deficient individuals may cause a severe, potentially lethal hemolytic anemia (favism) [3]. Griffiths and Ramsay, reported that vicine and convicine were first isolated from seeds of Vicia sativa and later found to be present in other Vicia species (faba, narbonensis, and sativa) included in the trial. Only trace quantities were found in the seed coat but concentrations of vicine ranging from 39 g kg^{-1} to 81 g kg^{-1} were detected in the radicle samples [42].

In a study in which the vicine, convicine, and L-DOPA changes were examined in two faba bean varieties according to their fruit development periods, it has been reported that the vicine and convicine contents were mostly in green cotyledons (moisture content of approximately 80%) when the moisture content of the seeds reached 40%, vicine and convicine contents a constant level (0.27-0.37%), and fruits have been reported to have no vicine and convicine contents [43]. In a study carried out to determine the content and the changes in vicine, convicine, and L-DOPA in faba bean seed germination, an increase in L-DOPA was observed while vicine and convicine decreased in cotyledons during germination [12]. No literature was found on the presence of these substances in faba bean flower, pollen, and honey. In the present study, it was determined that L-DOPA and vicine passed from flower to pollen and from pollen to honey. In this context, the present study is the first in the literature. In the present study, the highest vicine content (0.5 mg/g) was found in the flower sample. It was determined that 0.31% of this value passes to honey and the amount that passes to pollen (1.706%) was higher (Table 1).

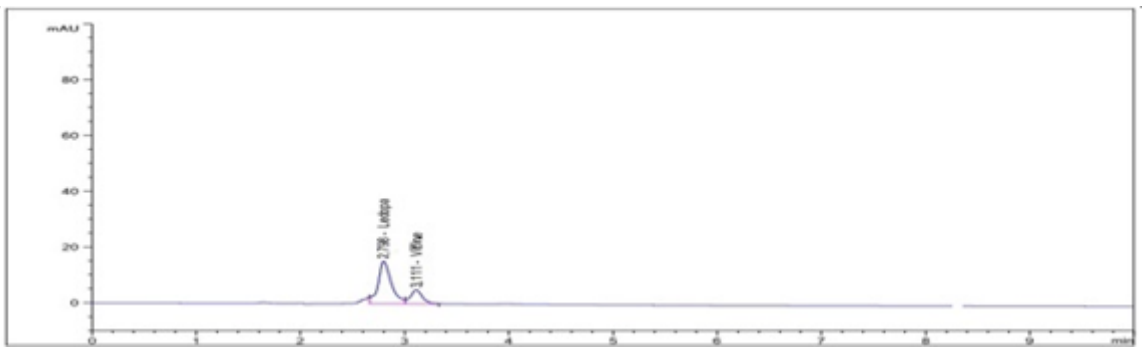

Figure 6. A. Standard Choromatogram of L-DOPA and Vicine

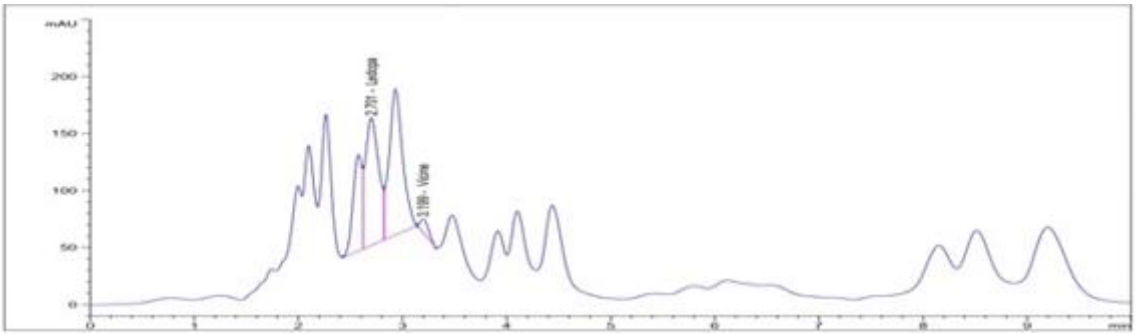

Figure 6. B. Choromatogram of Pollen Extract

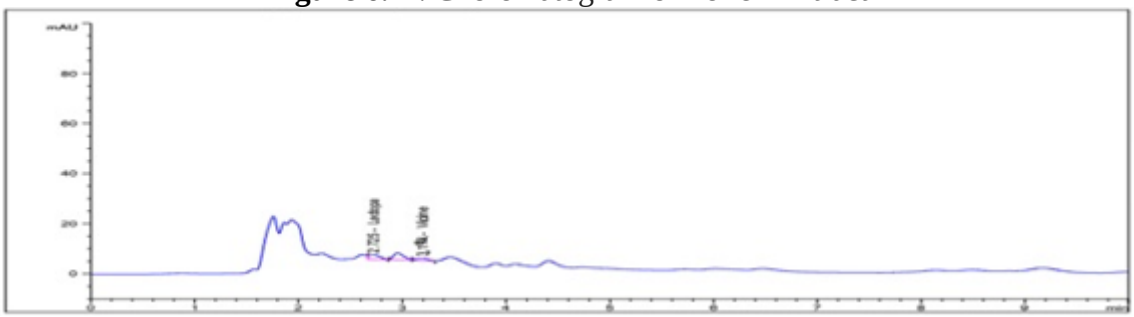

Figure 6. C. Choromatogram of Honey Extract

4. Conclusions

Turkey is located where edible and forage legumes were originated. Faba beans are one of these plants. However, faba bean agriculture is not as common as chickpeas or lentils. It is a plant that has the potential to be grown in a wider area due to the richness of its agricultural characteristics such as the possibility of being cultivated for winter in Turkey, the nitrogen fixation capacity and the potential of being a medicinal plant. One of the efforts to increase both the agriculture and consumption of the plant is to bring up different usage ways. Therefore, this study was carried out to show that the plant is the source of L-DOPA and the potential usability of flowers, one of the richest parts in terms of L-DOPA. According to the data obtained from the study, it was determined that the amount of phenol, flavonoids, L-DOPA and the amount of anti-nutritional vicine were the highest in the flower samples that are the source of honey and pollen. Regarding the bee products obtained from faba beans, the fact that there is no literature related to phenolic, flavonoid, L-DOPA and vicine compounds in pollen and honey makes this study significant. In the present study, the L-DOPA content of faba bean flowers was determined to be 4.23%. It was determined that 0.0758% of this amount passes to honey and 0.98% to pollen. According to the results, since flavonoid and phenolic substances are of vegetable origin, it can be argued that 60.9% of these substances which the bee takes from the faba bean pass to the product. Drug side effects can be reduced by using natural products containing natural L-DOPA instead of chemical-based drugs used in the treatment of Parkinson's patients. In this context, the study is of particular importance in terms of evaluating shed faba bean flowers at a ratio of 70%. Based on these data, we believe that the physiology of this transition, whether the products are used by the bee or not, and the ways to increase the level of these compounds will increase the importance of the plant.

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