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Spectrophotometric Analysis of Flavonoid Quantity in Pollen of Amygdalus communis L. and Determination of Biomarkers

Amygdalus communis L. Poleninde Flavonoid Miktarının Spektrofotometrik Analizi ve Biyomarkerların Belirlenmesi

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Abstract

In this study, the amount of flavonoid aggregates in the pollen of the common almond (*Amygdalus communis* L.) plant, which is common in Nakhchivan MR, was analyzed. The study of flavonoids in pollen can provide useful information for assessing the nutritional and healing quality of bee products. In the study, an extract was prepared on 60% ethyl alcohol from the pollen of the common almond plant. For the determination of flavonoids, a solution of 2% aluminum chloride in alcohol and a standard rutin solution were used. Biochemical analysis was performed by spectrophotometer method. The determination of flavonoid concentration was determined by measuring their absorbance at a wavelength of 310 nm. Based on the absorption results of solutions of different concentrations of rutin, the dependence in the calibration graph is expressed by the equation Y=0.032x+0.477. The correlation coefficient was R=0.993. The optical density (Y) of common almond (*A. communis* L.) pollen extract in alcohol was calculated based on the equation given in the calibration graph and was determined to be 0.041±0.02 mg/mL flavonoid (according to rutin). As a result of spectrophotometric research, it was determined that the pollen of common almond (*A. communis* L.) contains 10.29±1.16% flavonoid aggregate.

Keywords: Flower pollen, Flavonoid, Rutin, Spectrophotometry

Özet

Bu çalışmada Nahçıvan'da yaygın olarak bulunan badem (*Amygdalus communis* L.) bitkisinin polenindeki toplam flavonoid miktarı analiz edilmiştir. Çiçek polenindeki flavonoidlerin incelenmesi, arı ürünlerinin beslenme ve sağlığa yararlı özelliklerinin değerlendirilmesi için faydalı bilgiler sağlayabilir. Çalışmada badem bitkisinin poleninden %60'lık etil alkol kullanılarak ekstrakt hazırlanmıştır. Flavonoid tayini için alkolde %2 alüminyum klorür çözeltisi ve standart rutin çözeltisi kullanılmıştır. Biyokimyasal analizler spektrofotometrik yöntemLe gerçekleştirilmiştir. Flavonoid konsantrasyonunun belirlenmesi için 310 nm'de absorbans ölçümLeri yapılmıştır. Farklı rutin konsantrasyonlarına sahip çözeltilerin absorpsiyon sonuçlarına dayalı olarak, kalibrasyon grafiğindeki bağımLılık Y=0,032x+0,477 denklemiyle ifade edilmiştir. Korelasyon katsayısı R=0,993 olarak bulunmuştur. Badem (*A. communis* L.) poleni etanolik ekstraktının optik yoğunluğu (Y), kalibrasyon grafiğinde verilen denkleme göre hesaplandı ve 0,041±0,02 mg/mL flavonoid (rutine eşdeğer) olarak belirlendi. Spektrofotometrik yöntemle badem (*A. communis* L.) poleninin %10,29±1,16 toplam flavonoid içerdiği belirlendi.

Anahtar Kelimeler: Çiçek poleni, Flavonoid, Rutin, Spektrofotometri

1. INTRODUCTION

In an era of accelerated integration into natural products, the demand for bee products has increased even more in both the food industry and the pharmaceutical industry. For this reason, honey, propolis, wax, royal jelly, bee venom, bee bread, bee pollen, etc. the need for chemical analysis of bee products is increasing day by day. Despite the fact that these products are created from bioactive substances of both plant and animal origin, the quality and pharmacological properties of bee products are based on phytonutrients (Carpes et al., 2007; Degirmenci et al., 2020; Kolaylı & Keskin, 2020). Plants with a rich chemical composition are a source of essential components for the human and animal body.

Honey bees obtain their own food from nectar and pollen synthesized in the flowers of plants. Pollen, the first food of bee larvae, is created from pollen collected by worker bees. This shows that pollen has all the nutritional components necessary for the healthy and rapid development of the new generation. Flavonoids, especially synthesized in pollen, are evidence of the health of the bee family, the quality of honey to be obtained in the future and its pharmacological values. The flavonoid contained in pollen is considered biomarkers of bee products (Akbari et al., 2017).

Of great scientific interest is the study of the chemical composition of pollen, which is one of the main food products of bees. However, very little research has been done in this direction. The main goal of this study is to study the amount of flavonoid in the pollen of *Amygdalus communis* L., which is loved by bees. This plant, which blooms in early spring, is considered a rich source of food for bees due to its pollen and nectar (Guliyev, 2014).

2. MATERIALS and METHODS

2.1. Material

The material of the study was the pollen of the *A. communis* L. plant (Figure 1), which is distributed in the outskirts of the city in Nakhchivan Autonomous Republic. In 2023, the flowering dynamics of the almond plant in urban areas was observed to be from March 10 to March 17. The flowers were collected when the stamens were fully matured, the pollen was separated and dried in airy shade. Physico-chemical analyzes of the research were performed in the biochemistry laboratory of the Faculty of Medicine of Nakhchivan State University.

Figure 1. *A. communis* L.

2.2. Spectrophotometric Analysis

In the experiment, working solution, aluminum chloride solution and standard rutin solution were prepared according to the methodology mentioned in XI State Pharmacopoeia (USSR, 1987). The analyzes were performed using the spectrophotometer (BOECO Germany S-220) to determine the amount of flavonoids in the pollen of *A.communis* L.

2.3. Preparation of Extract

0.5 g (±0.001 g) of the dried pollen of the studied plant was weighed on an analytical balance and poured into a 50 mL flask. 15 mL of 60% ethanol was added to the pollen. The flask was connected to a counter-cooler and heated on a hot water bath (60-70 ºC) for 30 minutes. The extract was cooled at room temperature for 10-15 minutes and carefully filtered through a cotton-lined funnel (so that the sediment does not enter the funnel) into a 50 mL flask. The cotton used for filtering was placed in the extraction flask, 15 mL of 60% ethanol was added to it, and the extraction was carried out in the same manner 2 more times, and the obtained extracts were filtered into a volumetric flask. After cooling, the volume of the extract was brought to volume with 60% ethyl alcohol and mixed (solution A).

2.4. Determination of Flavonoid Aggregate

1 mL of solution A was poured into a volumetric flask with a volume of 25 mL, 2 mL of a 2% solution of aluminum chloride in 95% ethyl alcohol was added to it, and the volume of the flask was brought to volume with 95% ethyl alcohol. After 40 minutes, the optical density of the solution was determined.

Preparation of standard sample solution of rutin: 0.05 g (exact weight) standard sample of rutin is first dried at 130-135 ºC for 3 hours. It is dissolved by heating in 85 mL of 95% ethyl alcohol in a 100 mL volumetric flask. The solution is cooled, transferred to a 100 mL volumetric flask, the volume of the flask is brought to volume with 95% ethyl alcohol and mixed.

Preparation of rutin solutions for calibration chart: 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL of standard rutin (0.5 mg/mL) solution were poured into 25 mL flasks separately and 2 mL of 2% $AlCl_3$ solution was added to each one and brought to the mark with 95% ethanol (GOST R-55312-2012). A calibration graph was obtained based on solutions of different concentrations of rutin. Using a standard calibration graph, the total amount of flavonoids in the sample was calculated, and the results were reported as µg Rutin equivalents per gram of sample (µg Ru/mL). Quantitative determination by spectrophotometric method was performed in 3 repetitions. Then, the average value obtained was calculated. The metrological characteristics of the obtained average results were also analyzed by Statistical Package for the Social Sciences (SPSS).

3. RESULTS and DISCUSSION

To select the analytical wavelength, the absorption spectrum of the complex mixture of the standard solution of rutin and the aluminum chloride solution of *A. communis* L. was determined (Figure 2 and Figure 3).

Data file name	R1.WSC
Method	WL SCAN
Date & time	03.05.2023 15:57
Data Mode	ABS
Path length(mm)	0,100000001
Start WL(nm)	200
End WL(nm)	450

WL(nm)	Value
200	0,0969
210	0,1457
220	0,0845
230	0,2044
270	0,8953
280	1,4597
300	1,8968
310	1,9246
320	1,896
330	1,678
350	1,509
370	1,295
390	0,943
410	0,468
415	0,497
420	0,406
430	0,377
450	0,345

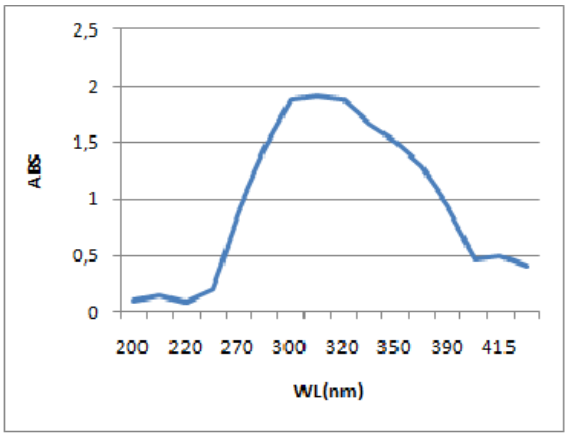

Figure 2. Absorption spectrum of complex of rutin with aluminum chloride C_R=40 µg/mL

Data file name	R WSC
Method	WL SCAN
Date & time	03.05.2023 16:15
Sample group name	
Data Mode	ABS
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End WL(nm)	450
WL(nm)	Value
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230	0,405
250	0,368
270	0,877
290	1,245
300	1,806
310	1,924
320	1,784
340	1,674
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400	1,109
420	0,785
450	0,469

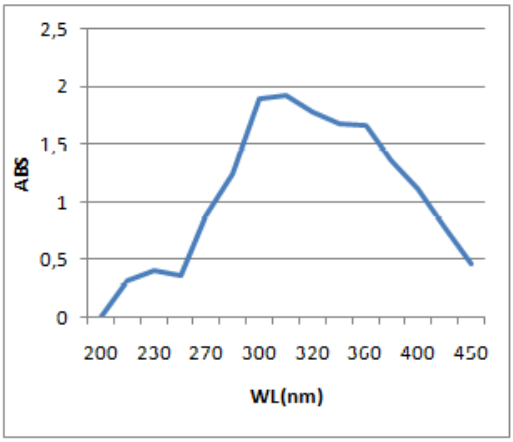

Figure 3. Absorption spectrum of the complex of the alcohol extract of *A. communis* L. pollen with aluminum chloride

Differential analysis of absorption spectra of the mixture of *A.communis* L. pollen and rutin extract in alcohol with aluminum chloride solution, it was determined that the absorption maximum in both solutions overlapped at 310 nm.

Determination of flavonoid concentration was performed by measuring the absorbance at 310 nm wavelength of the colored solutions resulting from the reaction between flavonoids and aluminum chloride. Based on the absorption results of solutions of different concentrations of rutin, a calibration graph was obtained. The reference was determined against ethyl alcohol. The dependence graph between the viscosity (X) and optical density (Y) of rutin solutions is expressed by the equation Y=0.032x+0.477. The correlation coefficient was R=0.993 (Figure 4).

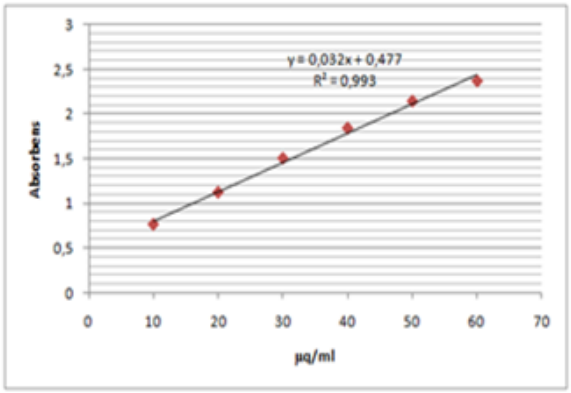

Figure 4. Calibration graph

Linearity in the range of 10-60 µg/mL routine was determined in the dependence graph. The optical density (Y) obtained in the alcohol extract of common almond (*A. communis* L.) pollen was calculated based on the equation given in the graph.The experiment was carried out in 3 repetitions and it was determined that the experimental solution contained 0.043

mg/mL, 0.039 mg/mL and 0.041 mg/mL flavonoids. The amount of flavonoid aggregate in percentage in the research raw materials was calculated using the following formula (Equation 1) (Vysochina et al., 2009).

$$X(\%) = \frac{C \cdot V_1 \cdot V_2 \cdot 100}{m \cdot V_3 \cdot 10^6} \text{ (Equation 1)}$$

The results obtained in the study were 10.78, 9.85 and 10.24%, and based on these results, metrological characteristics were analyzed statistically (Table 1).

Table 1. Metrological characterization of flavonoid quantitative results (n=3) in alcohol extract of common almond (*A. communis* L.) pollen

	T-Test One-Sample Statistics						
	N	Mean	Std. Deviation	Std. Error Mean			
A. communis L. pollen extract	3	10.2900	0.4670	0.2696			
	Test Value = 0						
	T	Df	Significance		Mean Difference	95% Confidence Interval of the Difference	
			One-Sided P	Two-Sided P		Lower	Upper
	38.163	2	<0.001	<0.001	10.2900	9.1299	11.4501

Calculations showed that the amount of flavonoid in common almond (A. *communis* L.) pollen was about 10.29±1.16%. The relative error in the measurements was determined to be 0.269%.

Recently, the attention of many scientists has been focused on the research of flower pollen. Because, this natural raw material is considered a source of protein, amino acids, vitamins, mineral substances and mainly also flavonoids. Flavonoids are antioxidant, antibacterial, antifungal, antiviral, anticancer, etc. has a wide range of effects (Rahman, 2007).

For this reason, the amount of flavonoids in plant pollen increases the pharmacological value of natural products (tea, bee products or pharmaceutical raw materials).

During the research conducted in this direction, analyzes of flavonoids in the pollen of most honey plants were found. It is known from the researches of Bakour et al. (2020) the amount of flavonoid in flower pollen of *Mentha spicata* (Lamiacea), *Anacyclus radiatus* (Asteraceae), *Calendula officinalis* (Lamiaceae), *Anethum graveolens* (Apiaceae) plants were calculated according to quercetin (QE). It was determined as 43±0.2 mg QE/g, 15.44±1.14 mg QE/g, 14.38±1.21 mg QE/g, 1.41±0.66 mg QE/g, respectively. The results of the research conducted by Okatan et al. (2021) on *Juglans* L. (walnut plant) cultivated in the Uşak region of Turkey showed that the concentration of flavonoids in the pollen of this plant varied from 1.53 to 4.12 mg QE/g based on dry weight. The total amount of flavonoids in *Juglans regia* L. pollen extract was 108.77 mg QE/g dry weight (Zurek et al., 2022).

4. CONCLUSION

In this study, the flavonoid content of common almond (*A. communis* L.) pollen distributed in Nakhchivan MR was investigated. It was determined that the components of bioactive substances of bee products mainly depend on the chemical composition of flower pollen. Biochemical studies of plant pollen create the basis for an in-depth study of the nutritional and healing properties of natural products, their application in apitherapy, their further development and use. Therefore, there is a need to conduct additional studies on flower pollen.

DECLARATIONS

Some part of this article was presented as a oral presentation in 1st International Apitherapy and Nature Congress (1-3 June 2023, Nakhchivan).

The authors declare that they have no conflicts of interest.

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