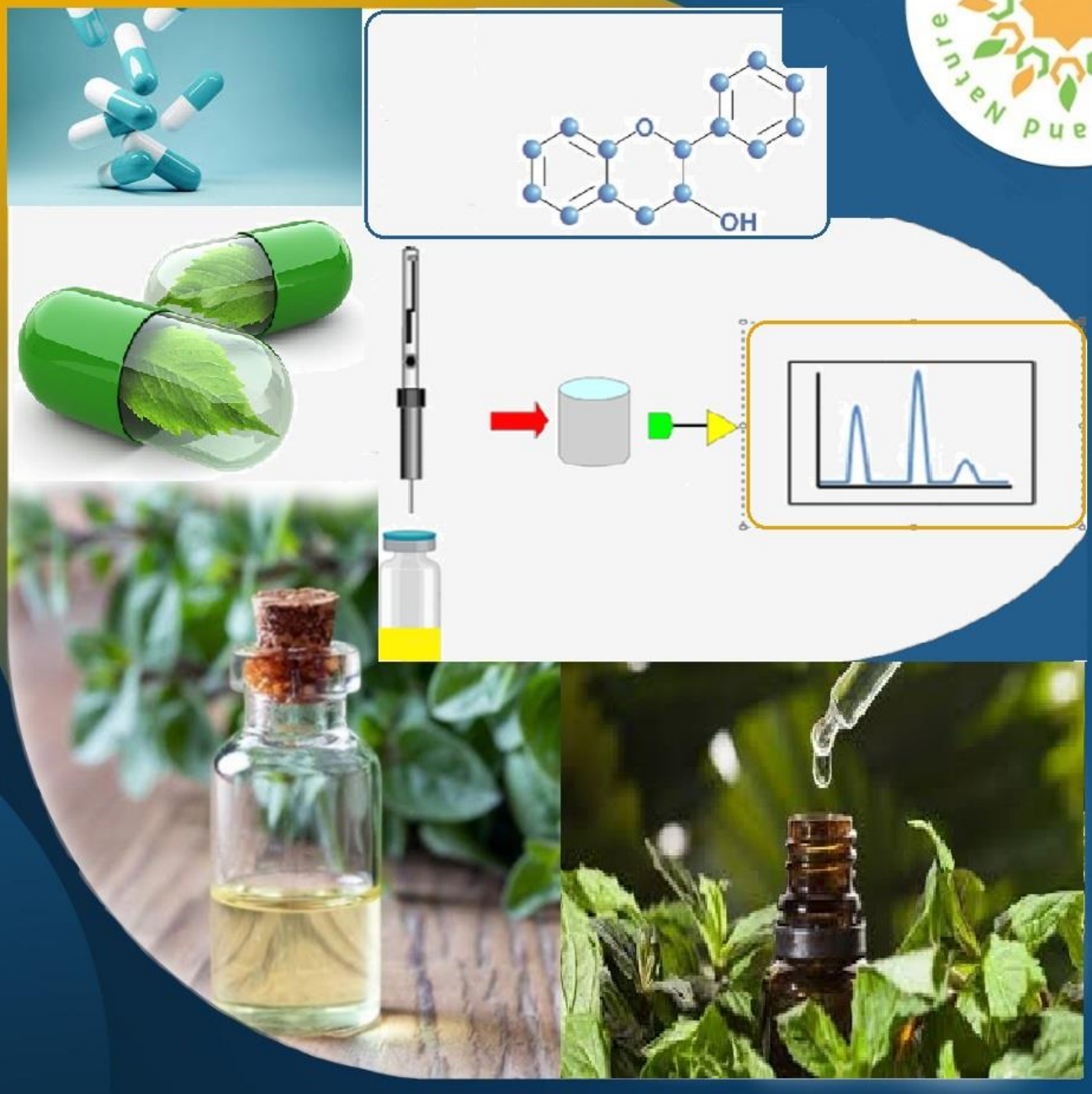
JUNE 2023

JOURNAL of APITHERAPY and NATURE

VOLUME 6 ISSUE 1



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www.apitherapyandnature.com
e-ISSN: 2667-4734

JUNE 2023

JOURNAL of APITHERAPY and NATURE

VOLUME 6 ISSUE 1

Publication Type: Peer-reviewed Scientific Journal

Publication Date: June 30, 2023

Publication Language: English

Published two times in a year (June, December)

www.dergipark.gov.tr/jan

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The Journal of Apitheraphy and Nature accepts English-language original articles, reviews, and letters to the editor concerning various fields of research. Main topics include:

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Analysis of Essential Oil Composition of Endemic Marrubium parviflorum subsp. oligodon Grown in Turkey by Using SPME with GC/MS

Türkiye'de Yetiştirilen Endemik Marrubium parviflorum subsp. oligodon'un Uçucu Yağ Kompozisyonunun SPME Kullanılarak GC/MS ile Analizi

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Received/Geliş Tarihi: 02/12/2022
Accepted/ Kabul Tarihi:13/02/2023
***Corresponding author** /Yazışılan yazar
Doi: 10.35206/jan.1213617
e-ISSN: 2667-4734

Abstract

The aim of the study is to determine the essential oil profile and antioxidant activity of endemic *Marrubium parviflorum* subsp. *oligodon.* The essential oil contents were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) using Solid Phase Microextraction (SPME). In addition, total phenolic content and the capacity of antioxidant activity measured by DPPH• and FRAP assays were determined. Twenty-three (64.12%) essential oil components were determined. Pentadecanolide was found to be the major essential oil and palmitic acid was determined as main fatty acid in *Marrubium parviflorum subsp. oligodon*. Total phenolic content was found to be 39.9±0.31 mg GAE/g sample. FRAP value was found to be 48.91±0.33 µmol Fe/g sample and DPPH• scavenging activity was found to be 0.76±0.03 mg/mL. This study is the first report in which the essential oil content of *Marrubium parviflorum subsp. oligodon* was determined by SPME.

Keywords: *Marrubium parviflorum* subsp. *oligodon*, SPME, Lamiaceae, Essential oil

Özet

Bu çalışmanın amacı, endemik *Marrubium parviflorum* subsp. *oligodon*'un uçucu yağ profilini ve antioksidan aktivitesini belirlemektir. Esansiyel yağ içerikleri, Katı Faz Mikro Ekstraksiyon (SPME) kullanılarak Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) ile analiz edildi. Ek olarak, DPPH• ve FRAP testleri ile ölçülen antioksidan aktivite kapasitesi ve toplam fenolik

madde miktarı belirlendi. Toplam 23 esansiyel yağ bileşeni (%64,12) belirlendi. *Marrubium parviflorum* subsp. *oligodon*'un toplam fenolik madde miktarı 39.9±0.31 mg GAE/g numune olarak bulundu. FRAP değeri 48.91±0.33 µmol Fe/g numune ve DPPH• süpürme aktivitesi 0.76±0.03 mg/mL olarak tespit edildi. Bu çalışma *Marrubium parviflorum* subsp. *oligodon*'un uçucu yağ içeriğinin SPME ile belirlendiği ilk rapordur.

Anahtar Kelimeler: *Marrubium parviflorum* subsp. *oligodon*, SPME, Lamiaceae, Uçucu yağ

Abbreviations: GAE, gallic acid equivalent; GC-MS, Gas Chromatography-Mass Spectrometry; SPME, Solid Phase Microextraction; FRAP, ferric reducing antioxidant power; DPPH•, 1,1-diphenyl2-picrylhydrazyl.

1. INTRODUCTION

Plants have been used for different purposes (fabric dyeing, treatment of diseases, essence, etc.) since ancient times. Today, plants constitute a large part of pharmaceutical raw materials. In recent years, the inadequacy of synthetic drugs produced against diseases and the presence of side effects have led people to use natural products. Therefore, the importance of medicinal and aromatic plants is increasing day by day (Ceylan, 2022). Turkey has a great potential in terms of having different climatic and ecological conditions and containing a large number of plant species and diversity (Şenkul & Kaya, 2017).

Essential oils are natural products obtained from the leaves, fruit, bark or root parts of plants. Although they are defined as oils because they do not mix with water, they are different from fixed oils (Ceylan, 1983). Essential oils have important properties such as antibacterial (Boyle, 1955), antiviral (Bishop, 1995), antioxidant (Sarıkurkcu et al., 2018), insecticidal (Karpouhtsis et al., 1998). Phenolic compounds are secondary metabolites produced by plants. These compounds have many properties such as anti-inflammatory, antimicrobial, antiviral, antimutagenic, anticarcinogenic, antiulcer, antioxidant effects (Balasundram et al., 2006; Moure et al., 2001).

Free radicals can occur as a by-product of normal reactions in our body, such as energy production, lipid degradation, or inflammatory processes. These free radicals play an important role in the development of various pathological conditions such as lipid peroxidation, DNA damage and cellular degeneration (Losada-Barreiro & Bravo-Diaz, 2017). Phenolic compounds and essential oils in plants are natural antioxidants and antimicrobials. These components have protective properties against diseases caused by free radicals. Therefore, people prefer herbal antioxidants instead of synthetic antioxidants due to their side effects (Ceylan et al., 2021).

The genus *Marrubium* L. belongs to the Lamiaceae family and is represented by about 28 taxa in the flora of Turkey. Some *Marrubium* species are used for different purposes in the world and in our country due to their medical and ethnobotanical importance. It is also preferred in beekeeping due to its abundant nectar and abundant flowers (Akgül et al., 2008).

Marrubium parviflorum subsp. *oligodon*, is a perennial herbaceous plant endemic to Turkey and grows in the Central Anatolia Region. It is also known as "mountain tea" among the people and is used as an antipyretic in the treatment of colds (Altundag & Ozturk, 2011; Sarıkurkcu et al., 2018).

The most widely used method to obtain essential oil from plants is hydrodistillation. Recently, the use of SPME (solid phase microextraction) method, which does not require solvent and is a faster method, has been increasing (Xing et al., 2019). In previous studies, hydrodistillation was used for essential oil analysis in *Marrubium* species. Therefore, in our study, it was aimed to determine the antioxidant activity and essential oil content of *Marrubium parviflorum* subsp. *oligodon* by SPME/GC-MS. The results obtained in this study can be considered as the first report in which the essential oil content of *Marrubium parviflorum* subsp. *oligodon* was determined by SPME.

2. MATERIALS and METHODS

2.1. Plant Material

The plant was collected from Nevşehir (Turkey) and identified by Dr. Mustafa Karaköse. Plant was dried at room temperature. Air dried sample was powdered in an electric grinder (Waring Commercial, USA). The dry plant sample was divided into two. 5 g of the dry sample was weighed and methanolic extract was prepared for antioxidant activity assay. The remaining sample was used for essential oil analysis.

2.2. Total Phenolic Content

Total phenolic content (TPC) of plant was obtained by utilizing the Folin-Ciocalteu assessment (Slinkard & Singleton, 1997). Standard was Gallic acid (different concentration) in this work. Shortly, 0.5 N 400 µL Folin-Ciocalteu solution, 20 µL methanolic samples (1 mg/mL), 680 µL of distilled water were stirred and the solution was vortexed. After 3-minute waiting, 400 µL of sodium carbonate (10%) was added and the solution was vortexed again. Then, the solution was left about 2 h. Following time, absorbance of the solution was determined to be 760 nm.

2.3. Determination of Antioxidant Activity

FRAP and DPPH$^{\bullet}$ assays were utilized in the calculation antioxidant activity. The FRAP assay is reduction of Fe^{3+}-TPTZ compound to the Fe^{2+}-TPTZ compound with electron giving material this situation (Benzie & Szeto, 1999). The 100 µL of sample solution or blank and 3 mL of FRAP solution (including TPTZ, iron (III) chloride and acetate buffer) were added and the solution was vortexed. The absorbance rates on 593 nm were determined to be about 4 min for at 25 °C.

The cleaning capacity of DPPH$^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) of metanolic extractions was defined utilizing the technique of Molyneux (2004). Various concentrations 0.75 mL of each sample extracts were vortexed together with 0.75 mL of a 0.1 mM of DPPH$^{\bullet}$ solution in methanol. After that, each extract was left at room temperature in the dark (50 min). Absorbance was monitored as 517 nm. Trolox was utilized as stock and amounts were explained as SC_{50} (mg sample per mL), the concentration of the antioxidant causing 50% DPPH$^{\bullet}$ scavenging.

2.4.SPME Procedure and GC/MS Analysis

GC analysis was performed on a Shimadzu QP2010 plus gas chromatography utilizing a TRB-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 µm). Shimadzu QP2010 Plus gas chromatography was connected to a Shimadzu QP2010 Ultra mass selector detector. One gram dry plant was added in a vial (10 mL), then the head space was placed to the solid-phase micro extraction apparatus (Supelco, USA). A polydimethylsiloxane/divinyl-benzene coating fiber was added in the head space. After at 50 °C with incubation time of 5 min, extraction was done at 10 min. Then fiber was inserted into the injection port of the GC-MS. The oven program was as follows: the first heat was 60 °C for 2 minutes, which was raised to 240 °C in 3 minutes, the last heat was kept at 250 °C for 4 minutes. The transporter gas was utilized as helium (99.999%) with a stable flux amount of 1 mL/minute. Detection was performed in electronic pulse range (EI); ionization tension adjusted to 70 eV and scanning range (40-400 m/z) was utilized to get mass. The volatile compounds were detected by comparison of their mass spectra of the two libraries (FFNSC1.2 and W9N11) (Renda et al., 2016).

3. RESULTS and DISCUSSION

Phenolic compounds are secondary metabolites produced by plants. They can be found not only in the fruit of the plant, but also in all parts of the plant such as leaves, roots and bark. These compounds protect the plant under different stress conditions such as drought, UV radiation,

pathogens and diseases. Phenolic compounds have many bioactive properties such as anti-inflammatory, antimicrobial, antiviral, antimutagenic, anticarcinogenic, antiulcer, antioxidant effects (Dietrich et al., 2004; Szajdek & Borowska, 2008).

Table 1 shows the results of total phenolic content, FRAP and DPPH analysis of *Marrubium parviflorum* subsp. *oligodon.* Total phenolic content was found to be 39.9±0.31 mg GAE/g sample. FRAP value was found to be 48.91±0.33 µmol Fe/g sample and DPPH• scavenging activity was found to be 0.76±0.03 mg/mL. Antioxidant activity analyzes performed in different *Marrubium* species in previous studies show diversity. Sarikurkcu et al. (2008) measured the antioxidant activity of *Marrubium parviflorum* subsp. *oligodon* using different solvents (hexane, water, methanol, ethyl acetate and dichloromethane). For the measurement of antioxidant activity, β-carotene-linoleic acid and DPPH• radical scavenging activity methods were used in the aerial part and essential oil of the plant. In addition, total phenolic and flavonoid were determined. While in total phenolic was high water extract (38.16 mg GAE/g), total flavonoid was high in methanolic extract (19.58 mg QE/g). Water extract was also found to be high in DPPH• scavenging activity. Our results are the second study examining the antioxidant activity of *Marrubium parviflorum* subsp. *oligodon*. There are also antioxidant activity studies with different *Marrubium* species in the literature. Total phenolic substance and DPPH radical scavenging activity were investigated in hexane and metanol extracts of *Marrubium parviflorum* and higher activity was found in methanolic extract (Yumrutaş & Saygıdeğer, 2010). In another study using different extraction solvents (petroleum ether, chloroform, ethyl acetate and n-butanol), it was reported that the DPPH• activity of *Marrubium parviflorum* Fisch. & C.A.Mey. was higher in ethyl acetate and n-butanol (Delnavazi et al., 2017).

Table 1. Result of total phenolic content and antioxidant activity

Sample	**Total phenolic content (mg GAE/g sample)**	**FRAP (µmol Fe/ g sample)**	**DPPH• (mg/mL)**
Marrubium parv.	39.9±0.31	48.91±0.33	0.76±0.03
Trolox	-	-	0.0025±0.11

Essential oils obtained from the leaves, fruit, bark or root parts of plants are of great interest in the pharmaceutical industry due to their biological potential (Kulaksız et al., 2018).

Species belonging to Lamiaceae family are used in the treatment of various diseases in traditional medicine and the *Marrubium parviflorum* subsp. *oligodon*, we used in our study, is an endemic species belonging to this family. Composition of the essential oils of *Marrubium parviflorum* subsp. *oligodon* was identified with SPME with GC-MS (Table 2).

Tablo 2. Essential oil composition of *Marrubium parviflorum* subsp. *oligodon*

Compound	**RI Exp.**	***Marrubium parv.* (%)**
Aldehyde		
Hexanal	804	0.80
Alcohol		
Hexanol	1034	9.83
Lauryl alcohol	2307	0.53
Ketones		
Acetoin	712	4.27
Civetone	2155	2.78
Hydrocarbons		
Dodecane	1205	0.72
Pentadecane	1269	0.92
Octadecane	1286	1.98
Tridecane	1299	0.68
Tetradecane	1406	3.01
Hexadecane	1459	0.55
Lactones		
δ-Undecalactone	1642	1.50
Pentadecanolide	1841	14.61
Terpens		
İsopulegol	1150	4.22
Linalool	1065	0.67
Neomenthol acetate	1599	0.78
Carboxylic acids		
Valeric acid	986	1.25
Caproic acid	1125	0.64
Esters		
Dietthyl malonate	912	0.53
Tetrahydrofurfuryl butyrate	1606	4.47
Fatty acids		
Palmitic acid	1949	6.72
Heptadecanoic acid	2103	1.61
Other		
Anethole	1290	0.52
Oxybenzene	988	0.53
Unknown		35.88
Total	**-**	**100**

Twenty-three (64.12 %) essential oil components were determined in this study. Pentadecanolide (14.61 %) and hexanol (9.83%) were determined as the main components. It is seen that palmitic acid (6.72%) is the main fatty acid.

When the results obtained are examined, it is seen that ketone, aldehyde, terpene, ester, carboxylic acid and hydrocarbon classes components are also detected in our sample. In previous studies, essential oil determination was made by hydrodistillation method in *Marrubium* species. The results obtained in these studies differ from our study. Bal et al. (1999) made the analysis of essential oil in *Marrubium parviflorum subsp. oligodon* and identified 139 compounds. Hexadecanoic acid (15.4%), germacren D (11.1%) and beta-caryophyll (10.0%) were indicated as the major components. Sarikurkcu et al. (2018) were determined 31 compounds as essential oil in *Marrubium parviflorum* subsp. *oligodon* and the main components were reported as (Z,Z) -farnesyl acetone (19.28%), caryophyllene oxide (15.85%), palmitic acid (15.4%) and pulegon (7.15%). In the essential oil study with *Marrubium parviflorum subsp. parviflorum* collected from Bingöl, β-caryophyllene (20.3%), germakren D (18.8%), bicyclogermacrene (10.2%) and spathulenol (7.3%) were found as the main components of the essential oil of the plant. In addition, fatty acid analysis was performed and palmitic acid (46.89%) and stearic acid ester (28.43%) were found to be high (Kılıç, 2018). It is seen that there is germakren D as a common component in studies of Bal et al. (1999) and Kılıç (2018). Sarikurkcu et al. (2018) also detected germakren D (3.85%), but at a lower rate. But, germakren D could not be detected in our study. However, the main fatty acid of palmitic acid in our study is similar to these studies. In addition, while pentadecanolide could not be detected in these studies, pentadecnolide (1.7%) was detected in the study of Khanavi et al. (2005) with *Marrubium parviflorum* Fisch. & C. A. Mey.

4. CONCLUSION

It has been thought that the extraction method, the region where the plant was collected and the harvesting time were effective in the differences between the results obtained in our study and the results in the literature. As we emphasized before, hydrodistillation method was used to determine the essential oil content in previous studies. In this study, SPME method was preferred for essential oil analysis. It can be used in different studies and compared with the hydrodistillation method, more volatile components can be detected in plants. As a continuation of this study, it is planned to determine the essential oil component of the same plant by using both SPME and hydrodistillation methods at the same time.

ACKNOWLEDGMENTS

This study was funded by the Scientific Research Projects Coordinatorship of Recep Tayyip Erdoğan University (Project no. FBA-2018-953).

DECLARATIONS

The authors declare that they have no conflicts of interest.

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The Effectiveness of Complex Treatment of Ulcerative Colitis in Integrative Medicine

Tamamlayıcı Tıpta Kompleks Ülseratif Kolit Tedavisinin Etkinliği

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Received/Geliş Tarihi: 02/05/2023

Accepted/ Kabul Tarihi: 20/06/2023

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Doi: 10.35206/jan.1319777

e-ISSN: 2667-4734

Abstract

The aim of the study was to develop a new complex treatment of ulcerative colitis using integrative medicine methods. The study was conducted on 16 patients with ulcerative colitis with total (8 patients) and left-sided lesions (8 patients). The progress of the disease was traced on the basis of complaints, levels of fecal calprotectin and lactoferrin, ESR and endoscopic picture of the colon mucosa. Depending on the severity and individual characteristics of the progress of the disease, the treatment lasted 2-8 months. The treatment was carried out against the background of taking mesalazine in two stages. At the first stage (1-3 weeks), treatment was carried out to eliminate the causes of the leaky gut syndrome (use of antiparasitic, antibacterial, antiprotozoal drugs) and restore the microbiota (use of eubiotics and short-chain fatty acids). The aim of the second stage of treatment (4-6 weeks) was immunomodulation and regeneration of the intestinal mucosa (the use of bioregulatory, isopathic, orthomolecular drugs and interleukin preparations). Patients received drugs orally, in the form of intravenous infusions and local injections into the metameric zones and acupuncture points of the large intestine. Also, in parallel, neural therapy was carried out with procaine at the points of the large intestine. The results obtained showed a significant decrease in calprotectin, lactoferrin and an improvement in the endoscopic picture of the large intestine. Colonoscopy showed complete recovery in group I in 2, and in group II in 3 patients, in other cases was noted 70% recovery of the mucous membrane. Thus, the use of a complex of integrative medicine methods with the use of safe bioregulatory drugs makes it possible to obtain high efficiency in the treatment of ulcerative colitis.

Keywords: Integrative medicine, Ulcerative colitis, Complex treatment

Özet

Bu çalışmanın amacı bütünleyici tıp metotlarını kullanarak ülseratif kolit için yeni bir kompleks tedavi geliştirilmesidir. Çalışma tüm kolonu tutan ülseratif kolitli 8 hasta, sol kolunu tutan 8 hasta olmak üzere toplam 16 hasta üzerinde yapıldı. Hastalıktaki ilerleme şikayetler, fekal kalprotektin ve laktoferrin düzeyleri, ESR ve kolon mukozasının endoskopik incelenmesi temelinde izlendi. Hastalığın ilerlemesinin ciddiyetine ve bireysel özelliklere göre tedavi 2-8 ay sürmüştür. Tedavi iki aşamalı mesalazin alımına karşı gerçekleştirildi. İlk aşamada (1-3 hafta) geçirgen bağırsak sendromunun nedenlerini ortadan kaldırmak (antiparaziter, antibakteriyel, antiprotozoal ilaç kullanımı) ve mikrobiyotayı yeniden yapılandırmak için (öbiyotiklerin ve kısa zincirli yağ asitlerinin kullanımı) uygulandı. Tedavinin ikinci aşamasının amacı ise (4-6 hafta) immünomodülasyon ve bağırsak mukozasının yenilenmesiydi (biyoregülatör, izopatik, ortomoleküler ilaç ve interlökin preparatlarının kullanımı ile). Hastalar kalın bağırsağın metamerik bölgelerine ve akupunktur noktalarına intravenöz infüzyonlar ve lokal enjeksiyonlar şeklinde oral yoldan ilaç aldılar. Ayrıca kalın bağırsağa prokain ile nöral terapi yapıldı. Kalprotektin, laktoferrin düzeylerinde anlamlı bir azalma ve endoskopik tabloda bir iyileşme sağlandı. Kolonoskopide grup 1'deki 2 hastada ve grup 2'deki 3 hastada tam iyileşme görüldü diğer vakalarda ise mukozada %70 iyileşme belirlendi. Güvenli biyoregülasyon ilaçlarının kullanımı ve tamamlayıcı tıp metotlarının kompleks kullanımı ile ülseratif kolit tedavisinde yüksek verimlilik elde edilmesini mümkün kılmıştır.

Anahtar Kelimeler: Tamamlayıcı tıp, Ülseratif kolit, Kompleks tedavi

Abbreviations: ESR, Eritrosit sedimentasyon hızı

1. INTRODUCTION

Ulcerative colitis is an idiopathic, chronic disease characterized by immune-mediated inflammation that occurs in the colon and rectum. It has long been recognized that ulcerative colitis begins in the rectum and usually spreads to part or all of the colon (Feuerstein et al., 2019; Ordás et al., 2012; Torres et al., 2012; Ungaro et al., 2017; Yan Sun et al., 2021). The cause of the aberrant immune response in this disease remains largely unknown, but dietary and environmental risk factors, as well as patient factors such as genetic predisposition and gut microbiota, play a role (Segal et al., 2021; Silverberg et al., 2005). It is important to note that the incidence and prevalence of ulcerative colitis is constantly increasing. The annual incidence is higher in industrialized countries and has been steadily increasing over the past decades worldwide (Asakura et al., 2009; Jones et al., 2019; Pasvol et al., 2020).

5-ASA preparations are the main medications for treatment for mild and moderate ulcerative colitis. In patients with an inadequate response to 5-ASA, is used immunosuppressive therapy. Despite therapeutic advances, treatment gaps still exist, only about 40% of patients with a short-term response to therapy maintaing clinical remission within a year (Hirten et al., 2018; Mao & Hu, 2016; Peyrin-Biroulet & Lemann, 2011; Robert et al., 2021).

In recent decades, there has been an increase in resistance to drugs used in the treatment of ulcerative colitis. Placebo-controlled studies using immunosuppressive drugs (tofacitinib, etrolizumab, infliximab, ustekinumab, etc.) showed a dose-dependent increase in the incidence of infections, especially viral and cases of thromboembolism (Sandborn et al., 2017; Sands et al., 2019; Vermeire et al., 2014; Vermeire et al., 2017). The above facts encourage the development of new approaches and methods for the treatment of ulcerative colitis.

Based on the foregoing, the aim of this study was to develop a new integrated approach to the treatment of ulcerative colitis using various integrative medicine therapies.

2. MATERIALS and METHODS

The work was performed in the clinic "Biological Medicine". The study was conducted on 16 patients with ulcerative colitis aged 15-45 years, divided into two groups: group 1 - 8 patients with total and group 2 - 8 patients with left-sided lesions of the large intestine. Patients with ulcerative colitis were diagnosed and grouped according to the Montreal classification (Lamb et al., 2019). General characteristics of patients are presented in Table 1.

Table 1. General characteristics of the examined patients with ulcerative colitis

Groups	Age years	Gender	
		Women	Men
Group 1 - patients with a total lesion of the large intestine	15-43	6	2
Group 2 - patients with left-sided lesions of the large intestine	24-45	3	5

The diagnosis of ulcerative colitis was based on clinical, laboratory, imaging, and endoscopic parameters. The course of the disease was traced on the basis of patient complaints, levels of fecal calprotectin and lactoferrin, ESR, CRP, and endoscopic picture of the colon mucosa. Calprotectin was determined by the immunofluorescent method on an express analyzer (CHROMA-2 Boditech Medince, South Korea), lactoferrin was determined by the enzyme immunoassay DRG (BIO SCREEN MS-500 BIOSAN, Latvia-England), and CRP was determined by the immunoturbulent method (ARCHITECT c 8000, Abbott USA). Endoscopic data were assessed by the presence of erythema in the colon, loss of normal vascular pattern, granularity, erosion, pseudo-erosion, friability, bleeding and ulceration using the PENTAX-Imagina EPK-i5500c apparatus, Japan.

Depending on the severity and individual characteristics of the flow patterns of the disease, the treatment lasted 2-8 months. The tactics and duration of treatment was determined by the degree of inflammation in the large intestine and was carried out in two stages while taking mesalazine. At the first stage, treatment was carried out to eliminate the causes of leaky gut syndrome (use of antiparasitic, antibacterial, antiprotozoal drugs) and restore the microbiota (use of eubiotics and short-chain fatty acids). The goal of the second stage of treatment was immunomodulation and regeneration of the intestinal mucosa (the use of bioregulatory, isopathic, orthomolecular drugs and interleukin preparations). Patients received drugs orally, in the form of intravenous infusions and local injections into the metameric zones and acupuncture points of the large intestine. Also, in parallel, neural therapy with procaine was carried out at the points of the large intestine.

The drugs used in the treatment were as follows:

At stage I

To eliminate increased intestinal permeability: Albendazole, Rifaximin, Bacteriophages, Metronidazole).

At stage II

For immunomodulation:

1. Isopathic therapy: Sanukehl Coli, Sanukehl Cand, Sanukehl Strep, Sanukehl Staph., Notakehl, Fortakehl (Sanum Kehlbeck, Germany)

2. Bioregulatory therapy: Mucosa comp., Traumel S, Belladonna HA, Pulsatilla HA (HEEL Germany)

2. Cytokine therapy: Anti-IL-1, IL-7, TGF beta (GUNA, Italy)

3. Orthomolecular therapy: Pascorbin 7.5g-50.0ml

4. Neural therapy: Novocain 1% 2.0-6.0 ml

5. Food supplements containing vitamins D, C, group B, zinc, curcumin, omega-3, butifara

For regeneration:

1. Chrysosan (Sanum Kehlbeck GmbH & Co. Germany)

2. Mucosa comp., Colon suis injeel, Graphites HA, Thuja injeel, Mercurius sublimatus corrosivus injeel (HEEL, Germany)

For restore the intestinal microbiota: Bifido- and Lactobacilli, Butyrate.

Drugs were administered orally, intravenously and locally.

Per os:

Mesalazine (1000 mg - 4000 mg / day, and 3 patients did not use mesalazine). If necessary, standard therapy also used glucocorticoids (prednisolone, metipred, solu-medrol, etc.) and monoclonal antibodies to TNF-α (azathioprine, infliximab).

For elimination causes of dysbiosis:

Sanukehl Coli, Sanukehl Strep, Sanukehl Staph., Notakehl (or together with Fortakehl). These drugs were used at a dose of 10 drops 3 times a day 30 minutes before meals.

For anti-inflammatory effect: Guna Anti İL 1 (the first 1-1.5 months during the period of exacerbation of the disease until the improvement of clinical symptoms, positive dynamics of inflammation indicators)

Guna IL7 (first 1-3 months and longer to increase intestinal IgA synthesis)

Guna TGF β (following 1-3 months and longer to improve regeneration).

The preparations were used 20 drops 2 times a day 30 minutes before meals.

Parenterally:

- **For bioregulation and immunomodulation** - Mucosa comp., Traumeel S, Belladonna HA, Pulsatilla HA (one ampoule of each IV every other day in one syringe).
- **Orthomolecular therapy** - Pascorbin 7.5 g - 50 ml IV drip every day, every other day, etc. depending on the severity of the course of the disease.

Locally:

- In the metameric zones of the intestine (Bioregulatory Segmental-Metameric Therapy) and acupuncture points (E 23,25,36; Rp12.13 (left),14,15; I6,7,9,10,12; Gi 4, 10 and eleven). Mucosa comp., Colon suis injeel., Graphites HA, Thuja injeel, Mercurius sublimatus corrosivus injeel, Chrysosan were administered 2 times a week, 5-15 procedures in total, depending on the severity of the disease.
- Neural therapy - Novocain 2% - 2.0 + 2.0 water for injection was injected into the acupuncture points Th9-L5 once a week (4-5 procedures in total).

3. RESULTS and DISCUSSION

The results obtained are presented in Table 2 and Figure 1. As can be seen from Table 2, after the complex treatment, there was a significant decrease in calprotectin, lactoferrin, ESR and CRP.

Table 2. Laboratory parameters in patients with ulcerative colitis after treatment (M ± m, n=16)

Indicators	Study timeline	
	Before treatment	After treatment
1st group (n=8)		
Calprotectin µg/g N <1 year <500 1-4 year <150 4-65 year <50 >65 year <100	246,8 ± 58,2	43,3 ± 19,9**
Min-Max	100-383	10,7-42
Lactoferrin µg/g N <7.25	179,3 ± 23,5	44,7 ± 17,7***
Min-Max	114,8-249,7	7,2-18,4
CRP µg/l N 0.0-5.0	18,12 ± 1,72	6,25 ± 0,36**
Min-Max	12,7-76	3-12,1
ESR mm/hour N >50 year 2-30 <50 year 2-20	20,75 ± 2.8	14,62 ± 3,2*
Min-Max	16-30	8-15
2nd group (n=8)		
Calprotektin µg/g N <1 year <500 1-4 year <150 4-65 year <50 >65 year <100	313,6 ± 70,1	18,12 ± 2,4***
Min-Max	100-383	10,7-30
Lactoferrin µg/g N <7.25	162,8 ± 28,32	37,4**
Min-Max	114,8-301	7,2-22
CRP µg/l N 0.0-5.0	22,88 ± 3,5	2,75 ± 0,36***
Min-Max	12,7-56,7	3-12,1
ESR mm/hour N >50 year 2-30 <50 year 2-20	37,.88 ± 14,46	6,25 ± 0,59**
Min-Max	16-30	8-15

Statistical significance compared to pre-treatment scores: *–p <0,05; **–p < 0,01; ***–p < 0,001.

In the first group of patients who had a total lesion of the large intestine after the course of treatment, the level of calprotectin decreased by an average of 82% ($p < 0.001$), lactoferrin by an average of 75% ($p < 0.001$, the content of CRP decreased by an average of 65% ($p < 0.001$), and ESR - by 29% ($p < 0.01$)). In the second group of patients who had a left-sided lesion of the large intestine in a comparative aspect with the first group, the decrease in the level of the studied parameters was more significant (table 2.). Thus, the level of calprotectin decreased by an average of 94% ($p < 0.001$), lactoferrin - by an average of 77% ($p < 0.001$), CRP - by an average of 88% ($p < 0.001$) and ESR - by an average of 83% ($p < 0.001$)). It should be noted that the duration of treatment in the first group compared with the second group was longer.

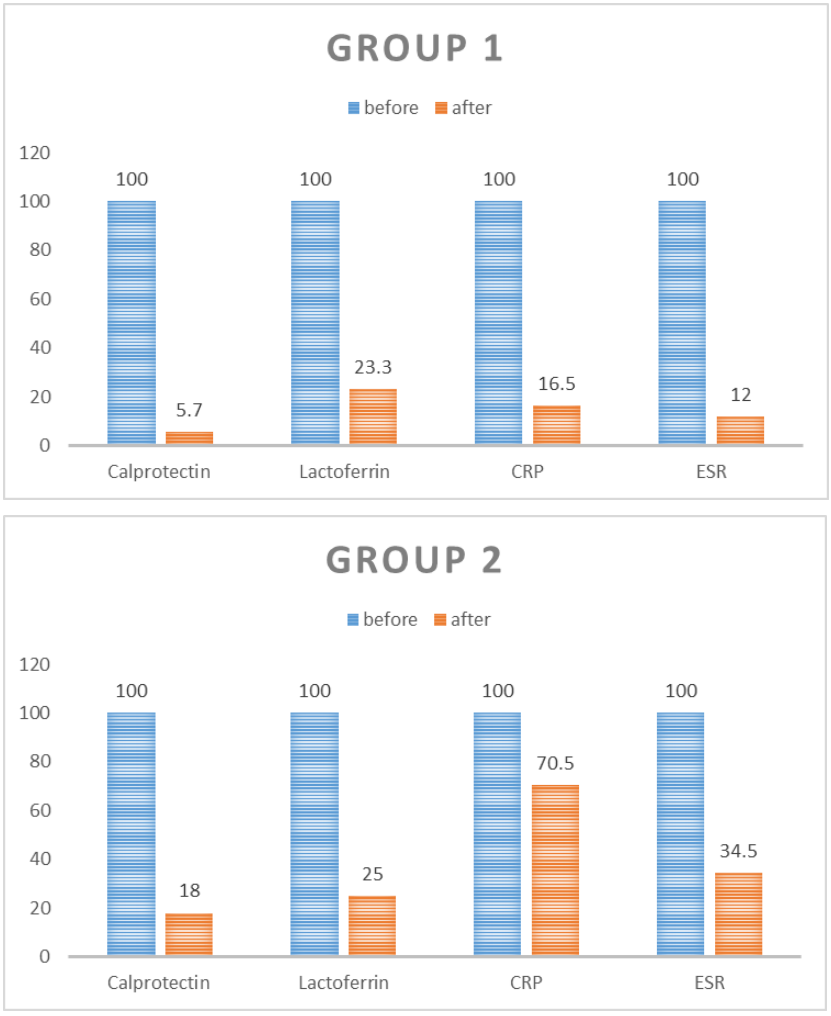

Figure 1. Dynamics of changes in laboratory parameters in patients with ulcerative colitis after treatment

The results of the colonoscopy performed before treatment were as follows. In patients with ulcerative colitis with a total lesion, the mucosa in all parts of the intestine during colonoscopy was sharply hyperemic, edematous, superficial ulcers were observed, prone to bleeding on contact, the veins in these places were obliterated in 7 patients (Figure 2). In 4 of

them, the mucosa bulged, having the appearance of a cobblestone pavement, in 2 patients were observed pseudopolyps. Repeat colonoscopy performed after improvement of clinical and laboratory parameters showed complete recovery in 2 patients with a total lesion; in two-restoration of the intestinal mucosa by 70% and single aphthous ulcers in separate areas.

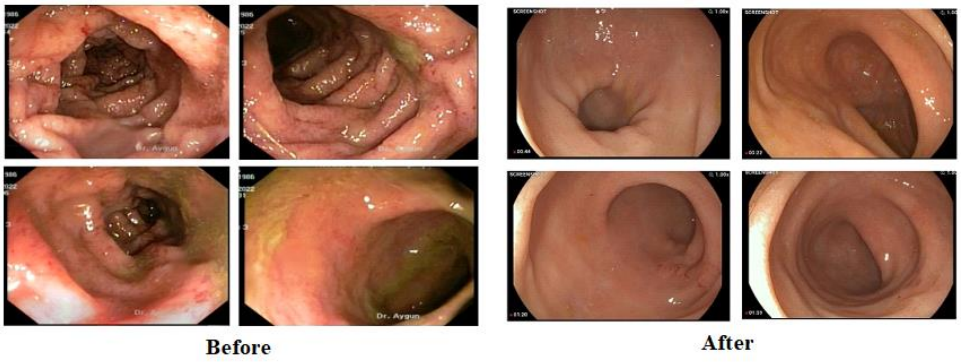

Figure 2. Colonoscopy picture in a patient of the 1st group before and after the treatment

In patients of the second group with ulcerative colitis with left-sided lesions, in 3 patients, starting from the distal transverse colon and in 4 patients, starting from the descending colon, the mucous membrane was sharply hyperemic and edematous, superficial ulcers were observed with a tendency to bleed on contact, veins in these areas were erased (Figure 3). In 2 patients, only the sigmoid colon was affected, and in one patient, only the rectum was affected.

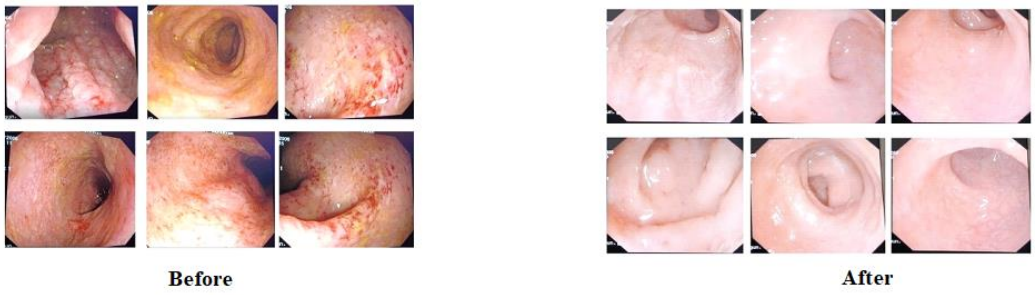

Figure 3. Colonoscopic picture in a patient of the 2nd group before and after the treatment

Repeated colonoscopy in this group also showed a pronounced improvement in the endoscopic picture of the large intestine after the treatment. In three patients with left-sided lesion, the colonoscopic picture was normal, in 2 patients healing was noted on most of the mucous membrane, in some places there were single superficial ulcers. Thus, in a comparative aspect, colonoscopy showed complete recovery in the first group in 2, and in the second group in 3 patients, in other cases, 70% recovery of the mucous membrane was noted in both groups of patients examined.

4. CONCLUSION

The complex use of bioregulatory, immunomodulatory, and regenerating drugs, along with standard therapy for patients with ulcerative colitis of varying degrees of colon damage, is highly effective and has a high level of safety. Causing adequate stimulation of nonspecific mechanisms of resistance and immunomodulation, the used drugs make it possible to influence on numerous pathogenetic links of this disease and provide repair of ulcerative defects of various depths in ulcerative colitis. An integrated approach to the treatment of the disease makes it possible to significantly optimize treatment and create new programs for the complex treatment of patients with ulcerative colitis.

DECLARATIONS

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

All authors declare that they have no conflicts of interest.

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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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Received/Geliş Tarihi: 08/05/2023
Accepted/ Kabul Tarihi:19/06/2023
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Doi: 10.35206/jan.1318471
e-ISSN: 2667-4734

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

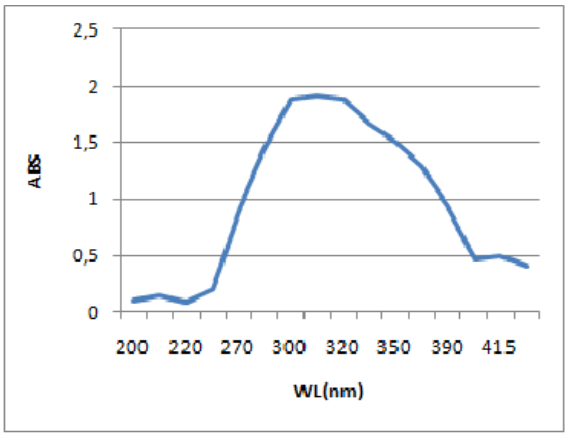

Figure 2. Absorption spectrum of complex of rutin with aluminum chloride C_R=40 µg/mL

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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210	0,324
230	0,405
250	0,368
270	0,877
290	1,245
300	1,806
310	1,924
320	1,784
340	1,674
360	1,657
380	1,356
400	1,109
420	0,785
450	0,469

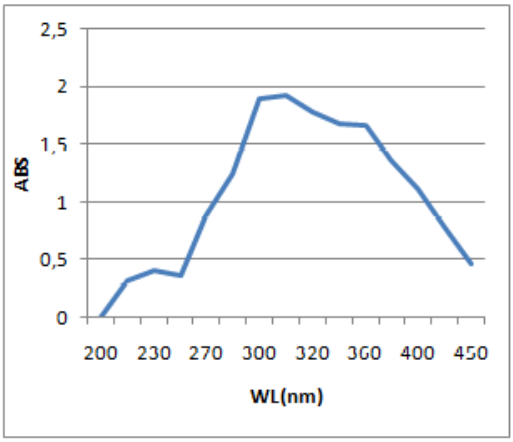

Figure 3. Absorption spectrum of the complex of the alcohol extract of *A. communis* L. pollen with aluminum chloride

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).

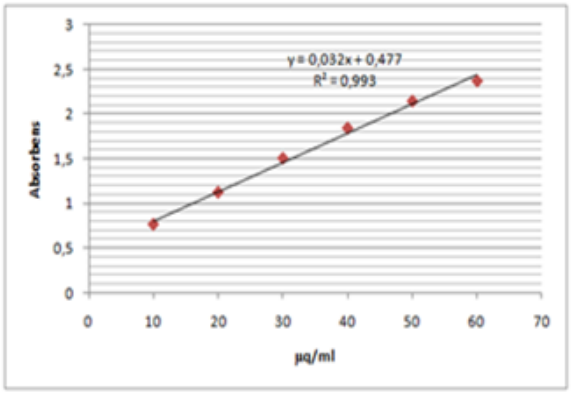

Figure 4. Calibration graph

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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