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Studies on the Composition and Biological Activity of Bee Pollen from Sivas Province

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Abstract

Bee pollen is an important nutrient for bees, especially for meeting the needs of adult and larval bees in terms of protein, vitamins, and minerals. Since the protein content of pollen meets the essential protein requirements for bees' nutrition, it should be collected in sufficient quantities for the hive and stored under appropriate conditions. Similarly, bee pollen has nourishing and therapeutic properties for human health. Various studies have been conducted in different countries to evaluate the physicochemical properties and determine the physicochemical composition of bee pollen, which is widely used in apitherapy, the pharmaceutical industry, the food industry, and the cosmetics industry. However, it has been observed that bee pollens obtained from hives can differ in terms of physical properties such as color and odor, as well as content profiles such as ash, moisture, cellulose, protein, sugar, carbohydrate, fat, vitamins, and minerals, depending on the climate, and floral structure conditions of the beekeeping region. Additionally, it has been found that the biological activity characteristics of bee pollen, as determined by various in vitro and in vivo studies, can also vary based on its content values. In this study, bee pollen samples were collected from certain districts of Sivas Province. The aim was to investigate the physical characteristics of the pollen samples, such as color properties, and to investigate their chemical contents like cellulose amounts, and their biological activity features, such as antioxidant activity. According to the results of the study, it was determined that bee pollens collected from different districts within Sivas Province showed differences both in terms of content and antioxidant activity.

Key Words: Antioksidant activity, Bee pollen, cellulose amount, Sivas.

Sivas İli Arı Polenlerinin İçerik ve Biyolojik Aktivite İncelemeleri

Özet

Arı poleni, arılar için önemli bir besin maddesidir, özellikle ergin, yaşlı ve larva dönemindeki arıların beslenmesinde protein, vitamin ve mineral madde gibi ihtiyaçlarının karşılanması için kullanılmaktadır. Polen içerisindeki protein oranı ile arıların beslenmesinde temel protein ihtiyacını karşıladığı için kovana yeterli düzeyde alınmalı ve uygun şartlarda depolanmalıdır. Aynı şekilde arı poleni insan sağlığı için besleyici ve terapötik özelliklere sahiptir. Apiterapide, ilaç sanayinde, gıda endüstrisinde, ve kozmetik sanayinde geniş bir kullanıma sahip arı poleninin, fiziksel- kimyasal özelliklerinin değerlendirilmesi, fizikokimyasal kapsamının belirlenmesi konusunda farklı ülkelerde çeşitli çalışmalar yapılmıştır. Ancak arıcılık yapılan bölgenin koşullarına, iklim ve floral yapısına göre, kovanlardan elde edilen arı polenlerinin renk, koku gibi fiziksel özellikleri bakımından ve kül miktarı, nem, selüloz, protein, şeker, karbonhidrat, yağ, vitamin, mineral gibi içerik profiller bakımından farklılıklar gösterdiği görülmektedir. Ayrıca arı polenlerinin çeşitli in vitro ve invivo çalışmalardan elde edilen biyolojik aktivite özelliklerinin de taşıdığı içerik değerlerine göre de değişebildiği görülmektedir. Bu çalışma ile, Sivas ilinin bazı ilçelerinden arı poleni örnekleri toplanmıştır. Polen örneklerinin renk özellikleri gibi fiziksel nitelikleri, selüloz miktarı gibi kimyasal içerikleri ve antioksidan etkinlikleri gibi biyolojik aktivite niteliklerinin araştırılması amaçlanmıştır. Çalışma sonuçlarına göre Sivas ili içerisinde farklı ilçelerden toplanan arı polenlerinin gerek içerik ve gerekse antioksidan etkinlikleri bakımından farklılıklar gösterdiği tespit edilmiştir.

Anahtar Kelimeler: Antioksidan aktivite, arı poleni, selüloz miktarı, Sivas.

Introduction

Today, the side effects of chemically produced drugs, the resistance of diseases to drugs and the rapid increase in diseases such as cancer, which are very difficult to treat, have increased people's orientation towards complementary and traditional medicine, including Apitherapy (Sorucu, 2019). In Türkiye, with the adoption of the “Regulation on Traditional and Complementary Medicine Practices” in 2014, Apitherapy courses and centers have started to be established (Sorucu, 2019; Ekici & Gölgeli, 2021). The desire to meet the food and food

supplements needed to maintain healthy life and well-being from natural and/or organic sources has led to a better understanding of the importance of beekeeping activities and increased interest in beekeeping and apitherapy. Although Turkey ranks first in the world in honey production, it lags behind in the production of other by-products of beekeeping and is not successful enough in their production (Burucu & Gülse Bal, 2017). Honey bees collect pollen from flowers and deposit it in pellets in pollen baskets (corbicula) on their hind legs to feed their young and young bees. Bee pollen is a mixture of these pellets. Honey bees moisten these pellets with their mouth secretions in order to compress them on their hind legs and to hold them together. These oral secretions contain enzymes such as amylase, catalase The pollen pellets also contain some nectar. The nectar is used to bring the pollen together (Silici, 2020). Protein and amino acids play an important role in bee nutrition and pollen is their only source of protein. Arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, isoleucine and valine, which are essential amino acids that honey bees need for growth and development, are present in pollen. The high quality of pollen increases the resistance of the honey bee against pathogens (Silici, 2020). The chemical, biological and physical properties of bee pollen vary depending on the plants from which it is collected, the soil characteristics and geographical location of the region where it is collected, climatic characteristics, the way it is collected and even its packaging (Silici, 2015; Akyol et al., 2015; Selamoglu et al., 2016).

Bee pollen can be recommended as a healthy and balanced nutrient with its sugar concentrations, essential amino acids, saturated and unsaturated fatty acids, Zn, Cu, Fe and high potassium/sodium ratios. Bee pollen has many biological activities such as antioxidant, anti-aging, antibiotic, antifungal, probiotic, anticarcinogenic, etc. depending on the source of collection (Sorucu, 2019; Dogan et al., 2014; Selamoglu et al., 2016). According to current literature, it has been determined that pollen eliminates the damaging effects of free radicals and some substances known to be carcinogenic with oxidant properties in the cell. It has also been shown that various pollen samples have antimicrobial, cell regenerating, appetite-enhancing effects and support the treatment of colds, osteoporosis, headaches and ulcers (Karlıdağ & Keskin, 2020; Hegazi, 2012). In clinical studies, it has been observed that it has the effect of regulating the functions of the intestines (Hegazi, 2012). It has also been found that pollen supplements used regularly contribute to the normalization of cholesterol and triglyceride levels in the blood (Hegazi, 2012; Aydın & Tekeoğlu, 2018). It has also been observed that bee pollen is effective in skin smoothing, moisturizing, spot removal, removal of wrinkles around the eyes and melanin index (Silici, 2020).

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Material and Method

Collection of Pollen Samples

Bee pollen samples were taken from beekeepers in Yıldızeli, Zara, Gürün, Divriği, Doğanşar, Hafik/Tozanlı and Merkez districts of Sivas province during the 2022 harvest period. The samples were brought to the laboratory and analyzed by taking the samples in appropriate storage environments.

Physical Evaluation of Pollen Samples

The physical properties of pollen such as shape and color vary according to the plant species from which they are collected (Özkök, 2018). The physical appearance and morphological shapes of pollen samples were examined under a microscope and photographs were taken.

Determination of Cellulose

Weigh approximately 1.0 g of pollen sample into a 250 mL beaker. Add 100 mL of 1.25% sulfuric acid solution and heat. After boiling, add 2-3 drops of antifoaming agent (silicone, amylalcohol, etc.) and boil for 30 minutes. To keep the volume constant during boiling, the beaker is covered with a cooling device (such as a 500 mm round bottomed balloon with cold water circulating in it) or a watch glass. After the end of the time, add 10 mL of 28% potassium hydroxide solution and boil for another 30 minutes. On the other side, a glass strainer is filled with quartz sand to a height of 8 - 10 mm. Before straining, the quartz sand is thoroughly moistened with hot distilled water and sucked with a water trumpet or vacuum pump to form a tight layer of quartz sand. The boiled sample is filtered hot through a prepared glass strainer. During the filtration process, raw cellulose particles may cause clogging. To prevent this, the vacuum is broken and the quartz sand layer is gently stirred with a glass baguette. The residue on the quartz sand layer is filtered twice with hot pure water, 10 mL of 1% sulfuric acid solution, hot pure water again, then 10 mL of 1% sodium hydroxide solution, hot pure water again and 10 mL of 1% sulfuric acid solution, and finally hot pure water twice more. Finally, it is washed again with acetone. During the different washing processes, the vacuum must be interrupted so that the crude cellulose residue can be well moisturized (Figure 1). After washing and filtering, the residue in the glass filter is dried in a drying cabinet at 130 ºC for 1 hour. After cooling in a desiccator, it is weighed. The weighed glass strainer is placed in the incinerator and burned at 550 ºC - 600 ºC for 30 minutes and weighed. Cooled in a desiccator and weighed.

Figure 1. Device used in cellulose determination

Antioxidant Activity Determination

Antioxidant, oxidant levels and oxidative stress indices of the isolates were determined using commercially available Rel Assay diagnostic kits with an accuracy rate of 99.9%. Total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the extracts obtained by different methods were evaluated with commercially available Rel Assay Diagnostic kits. (Erel, 2004; 2005). Trolox and hydrogen peroxide standard were used as reference for TAS and TOS analysis. In TAS analysis method, the change in absorbance at 660 nm depends on the total amount of antioxidants. Antioxidants in the sample reduce the dark blue-green ABTS radical to the colorless reduced ABTS form. The change in absorbance at 660 nm is related to the total antioxidant level of the sample. Fully automatic colorimetric measurement method is also used in TOS analysis method. Oxidants present in the reaction oxidize the iron ion to the iron ion-o-dianisidine complex. Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferrous ion. Oxidative Stress Index (OSI) is found by proportioning the values obtained from TAS and TOS analysis. As a matter of fact, there is a net oxidative stress capacity obtained depending on the total antioxidant capacity (TAS) and total oxidant capacity (TOS) provided by the plant extract.

Result and Discussion

According to the Pollen Communiqué of Turkish Standards Institute, pollen should have a distinctive color, smell and flavor. The shapes of pollen can vary greatly. When examined visually, they can be morphologically wrinkled, thorn-like protruding, oily, shiny, and sticky in appearance. The size of the pollen grains depends on the source plants from which the bee collects pollen, but generally the diameter should be between 10 µm - 100 µm. The diameter of the pollen pellets collected by the bee should be between 1 mm and 4 mm (Özkök & Sorkun, 2021; Demirci & Durna Daştan, 2022). They are generally roundish and can range in size from a minimum of 6µm to a maximum of 300µm (Knox, 1979). In terms of color, pollen can be in various colors and shades such as yellow, black, purple, blue, green, red (Figure 2). Below are photos of the bee pollen we collected (Figure 2).

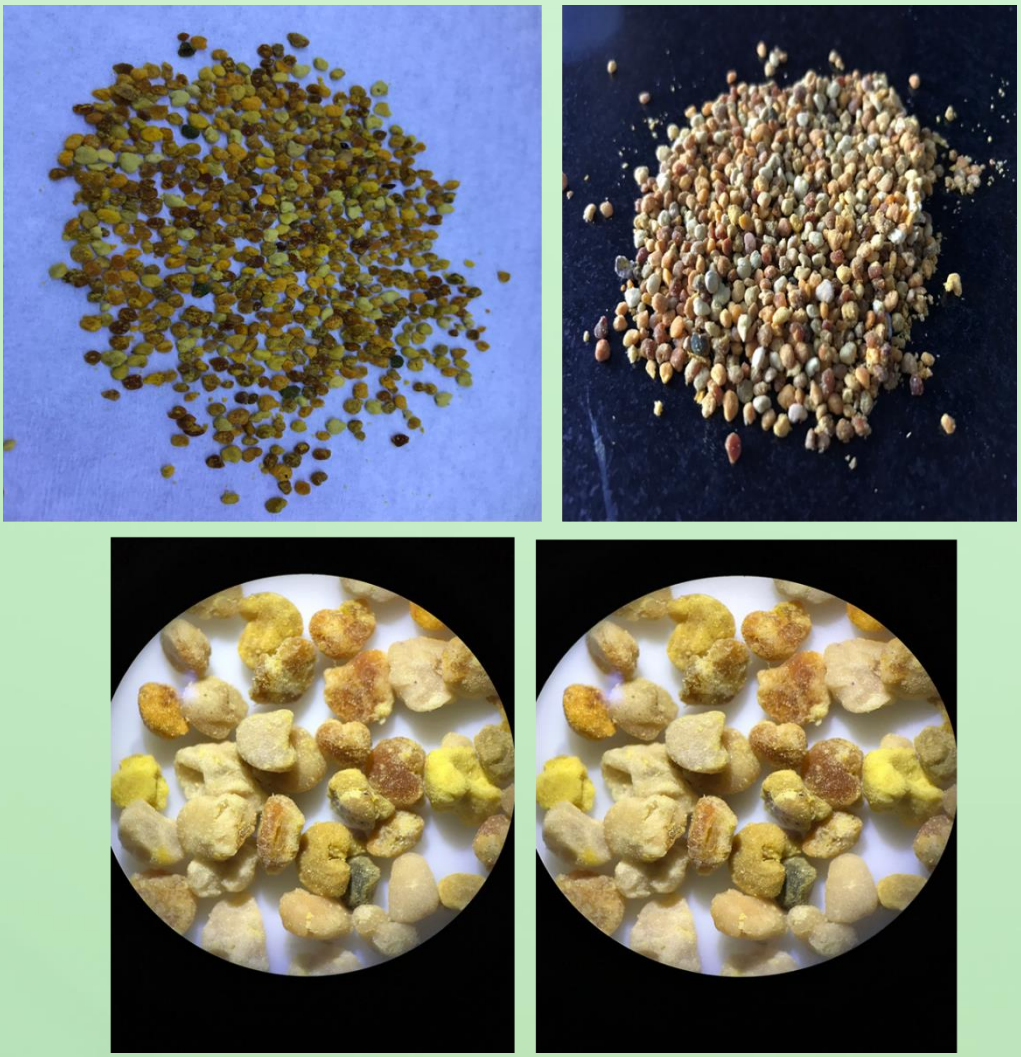

Figure 2. Photographs of some pollen samples collected

Bee pollen has been reported to be a very good source of antioxidants due to its bioactive components (Tutun et al, 2021). It has been stated that the main antioxidant components in bee pollen are carotenoids and phenolic compounds, and vitamins A, C and E in its structure also contribute as antioxidants (Asmae et al, 2021; Eşerler et al., 2023; Koçyiğit, 2021). The TAS and TOS values obtained for the determination of antioxidant and oxidant levels of pollen and the oxidative stress index (OSI) values obtained by proportioning these values to each other are given in Table 1.

Table 1. TAS, TOS and OSI values of pollen extracts (Values given as Mean ± SD).

Pollen sample	Extracts	TAS (mmol/L)	TOS(µmol/L)	OSI
Yıldızeli	water extract	5.0 ±1.0	2.1 ±0.4	0.04±0.01
	ethanol extract	9.2 ±2.0	2.6 ±0.5	0.03±0.01
Zara	water extract	6.6 ±1.3	2.5 ±0.6	0.04±0.02
	ethanol extract	9.6 ±1.5	2.1 ±0.5	0.02±0.01
Gürün	water extract	4.0 ±1.1	2.7 ±0.3	0.07±0.01
	ethanol extract	10.2 ±2.2	2.9 ±0.4	0.03±0.01
Divriği	water extract	6.9 ±1.5	2.5 ±0.5	0.04±0.02
	ethanol extract	10.8 ±1.4	3.1 ±0.7	0.03±0.01
Hafik-Tozanlı	water extract	7.5±1.7	4.5 ±0.7	0.06±0.02
	ethanol extract	11.4 ±1.6	4.1 ±0.6	0.04±0.01
City Center	water extract	7.3 ±1.1	4.9 ±0.7	0.07±0.03
	ethanol extract	9.3 ±1.0	5.7 ±0.8	0.06±0.02

The highest total antioxidant activity was obtained from the ethanol extract of pollen collected from Hafik region. In terms of antioxidant content, ethanol extracts generally showed higher activity. TOS values were very close to each other in all regions. In terms of OSI values indicating the oxidative stress load in pollen extracts, it is seen that pollen extracts collected from all regions have very similar values (Table 1). It has also been reported that bee pollen contains approximately 3.72% crude cellulose (Anđelković et al., 2012; Eşerler et al., 2023). According to TS 10255 Pollen Standard, the crude cellulose content in pollen should be above 0.9% (Anonim 2006; Eşerler et al., 2023). In this study, cellulose contents of bee pollen collected from different regions are given in the Table 2.

Table 2. Pollen cellulose contents

Pollen sample	Cellulose quantity
Yıldızeli	1.8%
Zara	1.6%
Gürün	2.0%
Divriği	2.2%
Hafik-Tozanlı	2.7%
City Center	2.4%

Conclusion

Standardization of bee products on international and national platforms is ongoing. Legislative work on bee pollen is also in progress and TSE standard no. 102551 is used to determine quality criteria (Özkök & Sorkun, 2021). Pollen with clearly defined properties such as total moisture content, total protein, total carbohydrates, etc. should be preferred by consumers and pollen with clearly defined content and quality criteria should be used by experts, especially in Apitherapy applications.

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