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Research Article

Phenolic Composition and Antioxidant Properties of Bee Pollens Belonging to *Drimia* spp. and *Castanea sativa* L.

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Abstract

Bee pollen is a nutrient-dense food and dietary supplement whose nutritional and bioactive properties are largely influenced by its botanical source. Its composition reflects the diversity of the plants from which they are collected, resulting in a rich array of essential nutrients, phenolic compounds, and other beneficial components that contribute to its value as a functional food. In this study, the biologically active molecules and antioxidant properties of two different bee pollen species from the Aydın and Kastamonu regions were investigated. Antioxidant markers, including total phenolic compounds, total flavonoids, ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) free radical scavenging activity, were measured. Additionally, 25 phenolic compounds were analysed using reversed-phase high-performance liquid chromatography (RP-HPLC-PDA). The total phenolic content was determined to be 27.3 mg GAE/g in chestnut (*Castanea sativa* L.) pollen and 8.5 mg GAE/g in Morca pollen *(Drimia* spp.). The findings revealed significant differences in both phenolic composition and antioxidant capacity between the two pollen species. The chestnut bee pollen *(Castanea sativa* L.) exhibited the FRAP, which is likely attributed to its high polyphenol content. Like chestnut honey and propolis, the strong FRAP of chestnut pollen confirms its high potential for use in apitherapeutic and nutraceutical applications.

Keywords: *Antioxidant, Bee pollen, Castanea sativa L., $DPPH^{\cdot}$, Drimia spp.*

Introduction

Bee pollen is the male gametophyte of angiosperms, collected by honeybees during foraging activities on floral structures. It adheres to the bees' hind legs, where it is mixed with nectar and digestive enzymes, forming granules that are then stored within the hive. This nutrient-dense substance supplies honeybees with vital nutrients for their growth and development, including proteins, lipids, sterols, vitamins, and minerals essential during the post-larval stage [1-3]. Bee pollen is also a highly nutritious food for humans, containing a range of macronutrients, such as proteins (10-40%), lipids (1-12%), carbohydrates (13-55%), and fibers (0.3-20%). It is rich in essential amino acids, fatty acids, B vitamins, carotenoids, and polyphenols, as well as sugars like fructose, glucose, and sucrose. Given its balanced nutrient profile and health-supportive properties, bee pollen is often used as a dietary supplement [4-6]. Studies have shown its benefits for immune function, potential cancer prevention, and protection against allergies, skin conditions, liver damage, and more. Furthermore, bee pollen intake has shown positive effects on reproductive health, neuroinflammation, and other conditions, making it a valued supplement for a wide range of health benefits [5,6].

The *Drimia* genus, belonging to the *Asparagaceae* family comprises several species known for their medicinal and ecological significance. Native primarily to the Mediterranean region, Africa, and parts of Asia, *Drimia* species thrive in arid and semi-arid climates. These plants are often characterized by bulbous structures and long, narrow leaves [7]. Many *Drimia* species, such as *Drimia maritima* (also known as sea squill), are rich in bioactive compounds like phenols and flavonoids, which have shown potenFt antioxidant and antimicrobial properties. Traditionally, *Drimia* spp. has been used in folk medicine, is locally called Morca (purple plant) in the Aegean region, and has purple pollen. This pollen, bee pollen, has non-toxic properties and is especially preferred for boosting the immune system. The bioactive composition of *Drimia* spp. is highly influenced by geographic and climatic factors, contributing to their diverse applications in pharmacology and nutraceuticals [7-9].

Castanea sativa, also known as the sweet chestnut, is a deciduous tree native to southern Europe and Asia Minor. Belonging to the *Fagaceae* family, this species is highly valued for its edible nuts, commonly known as chestnuts, which are rich in carbohydrates, vitamins, and minerals. *Castanea sativa* thrives in temperate climates and is often found in well-drained, slightly acidic soils across Europe, including regions of Türkiye, Italy, and France. Besides its nutritional value, sweet chestnut wood is also prized for its durability and is widely used in construction and furniture making. The tree's flowers provide an important nectar source for bees, resulting in honey with a distinctive, robust flavor, and the nuts themselves are enjoyed both as a staple food and in traditional culinary applications across Europe and Asia [10,11]. The chestnut tree *(C. sativa)* is highly valued for its diverse range of products, including its durable wood, edible nuts, and its medicinally significant honey, pollen, and propolis. Chestnut honey, in particular, is noted for its dark color and resistance to crystallization. It is distinguished by exceptionally high polyphenol levels, making it one of the most polyphenol-rich honeys worldwide. This rich polyphenolic content contributes to its potent antioxidant and antimicrobial properties, which have garnered attention in apitherapy therapeutic field utilizing bee products for health benefits. As a result, chestnut honey is not only a valued nutritional supplement but also a potential functional food with therapeutic applications [11].

Polyphenols are a diverse group of naturally occurring compounds known for their antioxidant properties, which help neutralize free radicals and protect against cellular damage. In bee products such as honey, propolis, pollen, and royal jelly, polyphenols contribute significantly to their health-promoting qualities. These compounds, especially flavonoids and phenolic acids, are associated with a range of biological activities, including anti-inflammatory, antimicrobial, and even anticancer effects [12]. Bee pollen is a natural product with a high protein content and is considered a functional food due to its significant phenolic compound composition. In addition to serving as a source of proteins, minerals, vitamins, and antioxidants for worker bees, it is also a valuable natural product with potential applications in apitherapy. Although bee pollen is rich in polyphenols, their composition varies depending on their botanical origin. Therefore, identifying bee products with high phenolic content, particularly bee pollens, could contribute to the discovery of novel sources for complementary medicine applications [13,14].

The objective of this study is to analyze and compare the phenolic profile and antioxidant properties of two distinct species of bee pollen. By establishing this comparative analysis, the study aims to provide a foundation for future research into the biologically active compounds and potential health benefits inherent to these two types of bee pollen.

Material and Methods

Samples and Extraction

The chestnut bee pollen used in this study was sourced in 2023 from experienced beekeepers in Kastamonu, Türkiye, while the Morca bee pollen was obtained from Aydın, Türkiye. The pollen samples, collected in dried form, were stored at +4°C to maintain their quality.

Ethanolic extracts of the bee pollen samples were prepared for total phenolic content (TPC) analysis. Approximately 3 g of bee pollen was mixed with 30 mL of 70% ethanol in a 50 mL falcon tube and stirred continuously at room temperature for 24 h using a shaker (Heidolph Promax 2020, Schwabach, Germany). The mixture was filtered first through coarse filter paper, then with a fine Whatman® membrane filter (Merck, Germany). The filtered extract was stored at -20°C for analysis, with one portion reserved for antioxidant testing and the other for phenolic profile analysis using liquid-liquid extraction [15].

Botanical Analyses

Microscopic analysis was conducted to verify the botanical origin of the bee pollen samples. This method was based on identifying pollen morphologies, following established protocols in the literature [16].

Determination of Total Phenolic Content

The TPC of the samples was assessed using the Folin-Ciocalteu method [17]. In brief, 20 µL of each extract was combined with 400 µL of 0.2N Folin-Ciocalteu reagent in a test tube and then diluted to 680 µL with distilled water. After a 3-min incubation, 400 µL of 7.5% sodium

carbonate (Na_2CO_3) was added to the mixture, followed by a 2-hour incubation at room temperature. Absorbance was subsequently measured at 760 nm using a UV-VIS spectrophotometer (Thermo Scientific Evolution TM 201, USA). TPC was calculated as mg gallic acid equivalents (GAE) per gram of sample based on a standard curve prepared with various gallic acid concentrations (0.031-1.0 mg GAE/mL).

Determination of Total Flavonoid Content

The TFC of the samples was determined following the method by Fukumoto and Mazza [18]. In this procedure, 0.25 µL of the sample extract was mixed with 50 µL of 10% aluminum nitrate ($Al(NO_3)_3$ and 50 µL of 1M ammonium acetate (NH_4CH_3COO). The mixture was diluted to a total volume of 3 mL with 99% methanol and incubated at room temperature for 40 minutes. Absorbance was then recorded at 415 nm against a blank. TFC was calculated as mg quercetin equivalent (QUE) per gram of sample using a standard curve, which was prepared with various concentrations of quercetin standards (0.031-0.5 mg QUE/mL).

Ferric Reducing Antioxidant Power (FRAP)

The FRAP of the samples were assessed using the ferric reducing antioxidant power (FRAP) assay, following the method of Benzie and Szeto [19]. For this, freshly prepared FRAP reagent was made by combining ferric tripyridyltriazine (Fe-III-TPTZ), iron(III) chloride ($FeCl_3$), and acetate buffer in a 40 mM HCl solution, along with 2.5 mL of a 20 mM iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) solution. The sample was prepared by mixing 3 mL of the FRAP reagent with 0.1 mL of the extract in a test tube, then incubating at 37°C for 4 min. Following incubation, absorbance was measured at 595 nm against a blank containing distilled water. A standard curve was created using various concentrations of iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$) ranging from 1000 to 31.25 mmol/mL.

Determination of Radical Scavenging Capacity (DPPH· Assay)

The radical scavenging capacity was determined by a simple method of DPPH [20]. The DPPH· radical was measured with the SC_{50} (mg sample/mL). For this, a 0.75 mL ethanolic extract was mixed with 0.75 mL of DPPH· radical. This mixture was incubated for 50 min in the dark at room temperature. After the incubation, absorbance was measured at 517 nm. The DPPH· capacity was measured using Trolox as a standard, and the results were expressed as SC_{50} (mg sample per mL).

Determination of Phenolic Profiles

Phenolic component analysis via RP-HPLC-PDA was performed following an enrichment step through liquid-liquid extraction. Initially, 10 mL of bee pollen extract was subjected to solvent evaporation at 40°C using a rotary evaporator. The dried extract was then redissolved in 10 mL of purified water, and the pH was adjusted to 2 using concentrated HCl. Organic phases from three successive extractions with diethyl ether and ethyl acetate were pooled. After complete removal of the solvents, the residue was dissolved in 2 mL of methanol, filtered through a 0.45 µm RC membrane filter, and injected into the HPLC system for phenolic analysis [15].

The phenolic profile of the samples was analyzed using a high-performance liquid chromatography (HPLC) system (Shimadzu LC-20AT, Shimadzu Corporation) equipped with a photodiode array detector (PDA) and a C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences). Standard calibration curves for 26 phenolic compounds were constructed based on absorbance readings at a wavelength of 250 nm. The mobile phase utilized was a mixture of 2% acetic acid in water (Phase A) and acetonitrile: water (70:30) (Phase B). Each sample and standard were injected at a volume of 20 µL, with the column maintained at 30°C and a flow rate set to 1.0 mL/min.

Statistical Analysis

The findings were calculated in Excel (Microsoft Corporation) using arithmetic means and standard deviation values from three repeated analyses. Since a limited number of data were used, no further statistical analysis was performed.

Results

Through this study, photographs of the bee pollen samples from two different botanical sources are presented in *Fig. 1*. The total phenolic and flavonoid content of the ethanolic extracts of the dried pollen samples are shown in *Fig. 2* and *Fig. 3*. The average total phenolic content (TPC) of the chestnut and Morca bee pollen was determined to be 27.3 mg GAE/g and 8.5 mg GAE/g, respectively.

It was observed that chestnut bee pollen has approximately three times higher phenolic compound contained than the Morca bee pollen. Similarly, when examining the total

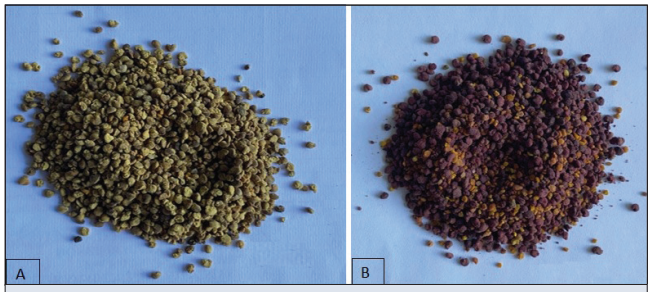

Fig 1. The *Castanea sativa* (A) and *Drimia* spp. (B) bee pollen samples

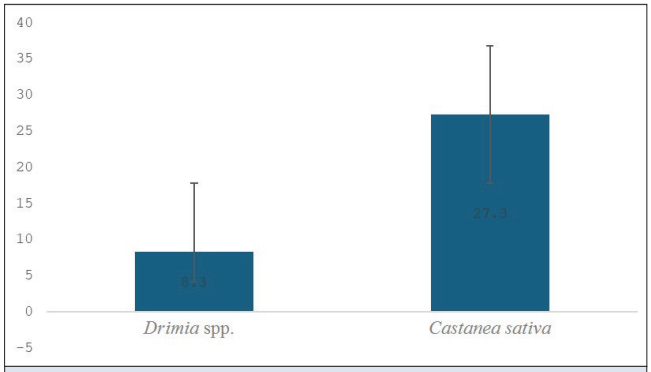

Fig 2. Total phenolic content (TPC) of the bee pollen samples

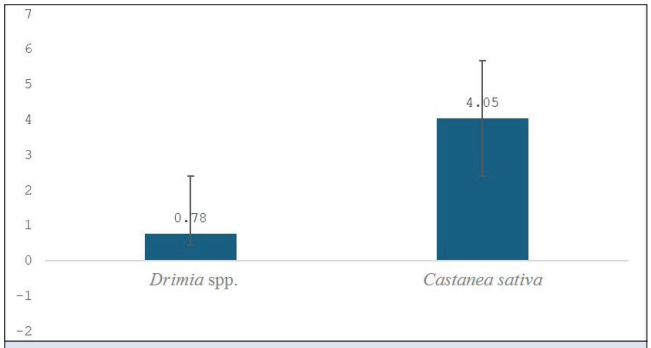

Fig 3. Total flavonoid content (TFC) of the bee pollen samples

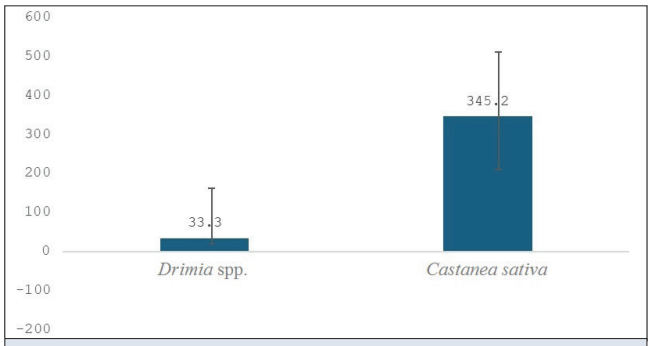

Fig 4. Ferric reducing antioxidant power (FRAP) of the bee pollen samples

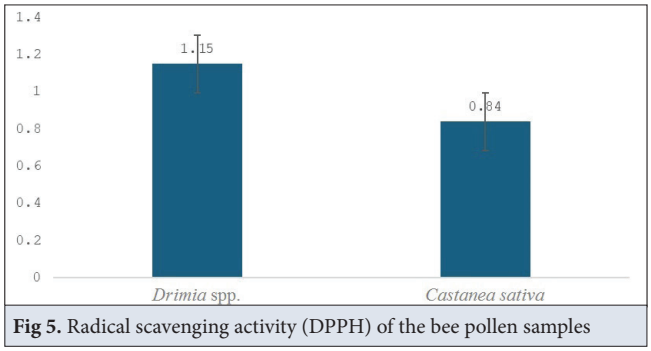

Fig 5. Radical scavenging activity (DPPH) of the bee pollen samples

flavonoid content, the average values were determined to be 4.05 mg QUE/g for chestnut bee pollen and 0.78 mg GAE/g for *Drimia* spp. It was found that chestnut pollen contains approximately five times higher levels of flavonoid compounds.

Table 1. *The phenolic composition of the bee pollen samples using HPLC-PDA*

Bioactive Compounds	**Phenolic Species**	***Drimia spp.***	***Castanea sativa* L.**
Phenolic acids (µg/100 g)	Gallic acid	-	-
	Protocatechuic acid	1095	-
	Chlorogenic acid	-	-
	p-OH Benzoic acid	404	1240
	Caffeic acid	-	-
	Vanillic acid	-	-
	Syringic acid		-
	p-Coumaric acid	10008	5420
	Ellagic acid	-	-
	Ferulic acid	-	-
	t-Cinnamic acid	3486	1250
Flavonoids (µg/100 g)	Catechin hydrate	-	-
	Epicatechin	-	-
	Rutin	-	640
	Myricetin	-	-
	Daidzein	-	-
	Luteolin	-	-
	Quercetin	-	-
	Naringenin	-	-
	Apigenin	-	-
	Hesperidin	-	-
	Rhamnetin	-	-
	Chrysin	6570	8480
	Pinocembrin	7160	3450
	CAPE	-	588
	Galangin	-	-

The current study analyzes the phenolic compositions of two pollen samples that were analyzed using RP-HPLC-PDA. The investigation was conducted based on 26 phenolic standards, previously validated for phenolic analysis. The data obtained are summarized in *Table 1*. The similarities and differences in common and unique components between the two bee pollen samples are summarized in the table. Protocatechuic acid was detected in significant amounts in the Morca bee pollen, while it was found to be below the detection limits in chestnut pollen. Similarly, rutin and CAPE were identified in chestnut pollen but were below the detection limits in bee pollen. On the other hand, p-hydroxybenzoic acid, *p*-coumaric acid, trans-cinnamic acid, chrysin, and pinocembrin were identified as common components in both pollen samples.

The antioxidant capacities of these two bee pollen samples were evaluated using two different methods. The results

are given in the *Fig. 4* and *Fig. 5*. The antioxidant capacities of these two bee pollen samples were evaluated using two different methods. In the FRAP (Ferric Reducing Antioxidant Power) assay, the capacity is measured by the pollen's ability to reduce the Fe (III)-TPTZ complex. A higher FRAP value indicates greater antioxidant capacity, with chestnut pollen exhibiting approximately ten times higher total antioxidant capacity than the other pollen sample.

Discussion

Bee pollen, a complex natural product obtained from the male gametophytes of flowering plants and collected by honeybees, plays a critical role in fulfilling the nutritional requirements of the colony and ensuring its survival. The phenolic profile, in particular, is considered a pivotal determinant of its biological activity and a reliable marker for the authentication of its botanical origin. Although widely utilized, inconsistencies in quality standards, along with insufficient knowledge regarding its bioavailability and long-term impacts, underscore the necessity for comprehensive scientific research to validate its potential applications in nutrition and medicine [1,5].

The biological quality parameters of honey, pollen and propolis are assessed based on their biologically active properties, with the total phenolic and flavonoid content measured as key chemical markers [21]. In this study, the total phenolic and flavonoid content in chestnut pollen was found to be approximately three times higher. A review of the literature indicates that chestnut pollen possesses significantly higher phenolic content compared to approximately nine other botanically sourced bee pollens [1]. Although the TPC value of Morca bee pollen was lower than that of chestnut pollen, it was found to have higher phenolic content than several other pollen types and excluding it from *Fagus* spp., *Rosa* spp., *Helix* spp., and *Rhododendron* spp. Given that chestnut pollen is distinct from all other pollen types, excluding it from comparison, it can be concluded that Morca bee pollen also contains a significant number of phenolic compounds. Comparing the TPC values of these two bee pollen types with reported values for bee bread in the literature, it was found that the phenolic compound content in bee pollen is approximately 3 to 5 times higher than that in bee bread [2,22]. Furthermore, when the phenolic content of these pollen samples was compared with that of strawberries and figs, it was observed that both pollen types contain higher polyphenol levels than either fruit [13,23].

The total antioxidant capacities of these samples were measured using ferric reducing antioxidant power (FRAP). In the assay, the capacity is measured by the pollen's ability to reduce the Fe(III)-TPTZ complex. A higher FRAP value indicates greater antioxidant capacity, with chestnut pollen exhibiting approximately ten times higher total antioxidant capacity compared to the other pollen sample.

The other antioxidant test was DPPH· radical scavenging activity, that the values were found related with FRAP results. The results, expressed as SC_{50} values, represent the amount of extract needed to scavenge 50% of the DPPH· synthetic radical. A lower SC_{50} value indicates higher radical scavenging activity, with chestnut pollen exhibiting a lower SC_{50} value, demonstrating greater activity. The antioxidant properties of pollens are largely attributed to their polyphenol content, making this outcome expected. However, while chestnut pollen showed an FRAP value approximately ten times higher, its DPPH· was only about twice as high, suggesting that polyphenols alone may not fully explain this result. The high DPPH· capacity in Morca pollen is likely due to other antioxidant compounds present, potentially including non-polyphenolic antioxidants [24]. A study reported that extracts obtained from *Drimia maritima* flowers exhibit strong antioxidant, anti-inflammatory, and photoprotective activities [25].

In Morca bee pollen, *p*-coumaric acid was identified as the major phenolic acid component, along with the presence of protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and t-cinnamic acid. Among the flavonoid compounds analyzed, only chrysin and pinocembrin were detected in Morca pollen. In chestnut pollen, chrysin, pinocembrin, and *p*-coumaric acid were found at major levels, while p-hydroxybenzoic acid, cinnamic acid, rutin, and CAPE was present in smaller quantities. Rutin, CAPE, and epicatechin were exclusively detected in chestnut pollen. In a study similar to ours, chestnut pollen was reported to be rich in p-hydroxybenzoic acid, rutin, and cinnamic acid [1,2]. Extracts obtained from *Drimia maritima* flowers have been shown to be rich in coumaric acid, ferulic acid, cinnamic acid, gallic acid, *p*-coumaric acid, chlorogenic acid, and salicylic acid [25].

As with chestnut bee pollen, chestnut honey and chestnut propolis, the unique biological value of chestnut-derived products is well-documented, and their significance as apitherapeutic products is widely acknowledged. Research conducted on chestnut flowers has demonstrated their abundance in chestnut pollen, and that aqueous extracts of these flowers exhibit elevated antioxidant and antimicrobial properties [26,27].

In conclusion, this study found that bee pollen contains significant amounts of polyphenols, with the quantity of these compounds varying depending on the botanical origin of the pollen. Chestnut pollen was identified as a product with remarkably high biological activity. However, it was also determined that Morca pollen *(Drimias* spp.), while not as potent as chestnut pollen, possesses a

noteworthy antioxidant capacity. Conversely, research on chestnut pollen has been conducted; however, this study is the inaugural one to present Morca bee pollen *(Drimia* spp.) in the scientific literature.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author (M. Maharramov) upon reasonable request.

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Conflict of Interest: There is no conflict interest.

Declaration of Generative Artificial Intelligence: I declare that the article, tables and figures were not written by artificial intelligence and artificial intelligence-supported technologies.

Author Contributions: CB: Methodology and HPLC analyses, FYK: Antioxidant analysis, EA: Designing an article and botanical analyses, BM: Statistical analysis, MM: Designing the article. All authors have read and agreed to the published version of the manuscript.

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