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BEE POLLEN: CHEMICAL COMPOSITION AND POTENTIAL BENEFICIAL EFFECTS ON HEALTH

Running title: **Bioactive Components of Bee Pollen**

Abstract

Since long ago, bee pollen has gained reputation as an important source of energy and beneficial substances from the nutritional and health points of view. However, in the recent years this natural product has gained much interest, either from the scientific community, increasing the number of works about it, or from the general population, that buy it as natural functional food. This review intends on one hand to focus on the chemical composition of the bee pollen, since it is the chemical components that provide it with beneficial properties. On the other hand, it also addresses the health effects of this product, by referring the different studies that have been done and published about this theme. From the information found on the literature it was possible to depict some of the most investigated effects of bee pollen, such as antimicrobial, anti-inflammatory, antioxidant and antimutagenic activities.

Keywords: Bee pollen, *Apis mellifera*, phenolic compounds, fatty acids, antimicrobial, anti-inflammatory, antioxidant.

1. INTRODUCTION

Bee pollen derives from the joining of flower pollens with nectar and salivary substances of the honeybees (*Apis mellifera*) [1]. In their collecting excursions the bees gather pollen grains from the flowers into pollen capsules on their back legs by means of numerous filaments [2]..

Bee pollen is used in the hive to feed the bees in all their stages of development. In particular, the particles of pollen constitute the most significant source of proteins to guarantee the survival of the bees [2].

Inside the hive, the pollen is stored separately from the nectar cells. The collection of this natural product is based in scraping pollen off of the bees' legs as they enter the hive [3]. A trap is installed in front of the entry to the hive, and when the worker-bees come home they let their pollen pellets fall in the trap, being then reserved in a container [1].

Bee pollen constitutes one of nature's most complete and nutritious foods because it gathers almost all nutrients necessary for humans. Bee pollen is a complete sustenance and contains many components not found in foods from animal origin, being also richer in proteins. For example, for equal amounts, bee pollen is richer in amino acids when compared to beef, eggs, or cheese. Furthermore, around half of the proteins are present as free amino acids, and these are available for direct use by the body. One single teaspoon of pollen corresponds to the amount gathered by one bee working during eight hours per day for one month, and it contains more than 2.5 billion grains of flower pollen. The bee pollen has been called "life-giving dust" and has been used in folk medicine due to its wide range of therapeutic properties. According to Medeiros et al. [4], bee pollen is used in folk medicine to alleviate colds, ulcers, anaemia and allergies. Other health effects include skin problems (dry skin, babies' diaper rash or eczema), infertility, asthma, addiction in general and alcoholism in particular or problems of the stomach. Some herbalists acclaim that bee pollen enhances athletic performance and also minimizes adverse effects of chemotherapy. Furthermore, bee pollen plays an important role on a diet aimed at controlling and stabilizing weight because it could correct a likely chemical imbalance in body metabolism which may be associated with unusual gain or loss of weight.

It is important to mention that the German Federal Board of Health has officially recognized bee pollen as a medicine [5].

Bee pollen consumption has some tradition among American and Indian cultures, but not in Europe. However this reality is changing and more and more interest is demonstrated by European consumers. Bee pollen in grain is available at many health food stores and it can also be found in other natural dietary supplements.

2. CHEMICAL COMPOSITION OF BEE POLLEN

The pollens collected by the bees are rich in proteins, free amino acids, carbohydrates, lipids, vitamins (including B-complex and folic acid) and minerals. Bee pollen is said to be the “only perfectly complete food”, because it comprises all the essential amino acids that the human body necessitates. Besides, it also contains bioactive compounds, which, even if present in small quantities, play an important role on human health. For example, bee pollen contains carotenoids, phenolic compounds and in particular flavonoids, which are products that results from the metabolism of plants and which possess numerous phenolic compounds, and that have antioxidant activity [3,4].

However, many factors can contribute for the composition of bee pollen, such as the plant source, the climate and weather conditions, the characteristics of the soil, as well as the actions of the beekeeper [6,7].

The major components of bee pollen are carbohydrates (ranging between 13% and 55%), crude fibres (0.3% to 20%), proteins (10% to 40%) and lipids (1% to 10%) [1]. The data about the general composition of bee pollen used to be enough for the majority of purposes, however, presently the utility of specific data on the composition of bee pollen is progressively being acknowledged [8]. In this way, many recent studies focused on specific pollens and studied also the bioactive compounds apart from the basic chemical constituents.

According to Villanueva et al. [9] bee pollen is of great importance, providing a good complement of the diet due to the its richness in proteins, and particularly in fats and carbohydrates.

Table 1 summarizes some studies found in literature about the chemical composition of bee pollen, which will be analysed in more detail along the present chapter.

Table 1. Studies about the chemical composition of bee pollen.

	General chemical composition	Sugars	Proteins and amino acids	Fatty acids	Phenolic compounds	Vitamins	Others
Almeida-Muradian et al. [2]	•						
Arruda et al. [10]						•	
Arruda et al.[11]						•	
Avni et al. [12]				•			
Čeksterytė et al. [13]				•			
Coronel et al. [14]	•						
Di Paola-Naranjo et al. [15]					•		
Fanali et al. [16]					•		
Feás et al. [3]	•			•	•		
Human and Nicolson [17]	•		•	•			
LeBlanc et al. [18]					•		
Marchini et al. [19]	•						
Morais et al. [7]					•		
Pascoal et al. [1]					•		
Qian et al. [20]		•					
Šarić et al. [21]					•		
Silva et al. [22]							•
Szczęsna [23]			•				
Szczęsna [24]				•			
Vit and Santiago [25]	•						
Xue et al. [26]							•

2.1. General chemical composition

Feás et al. [3] studied twenty two samples of organic bee pollen harvested in the Douro International Natural Park (Portugal). The samples evaluated included nine botanical families: *Cistaceae*, *Boraginaceae*, *Rosaceae*, *Fagaceae*, *Asteraceae*, *Fabaceae*, *Ericaceae*, *Mimosaceae* and *Myrtaceae*. The average values of the physico-chemical and nutritional properties including also energy were: 67.7% carbohydrates, 21.8% crude protein, 5.2% crude fat, 2.9% ash and 404.3 kcal/100 g.

Marchini et al. [19] determined the physico-chemical composition of pollen samples collected in State of São Paulo, Brazil, from 5 beehives of Africanized *Apis mellifera*. Their results showed that the pollens had, on average, 21.5% proteins; 2.8% ashes; 23.6% moisture; 76.3% of dry residue; 3.5% lipids; 28.4% of total sugars; 20.7 mEq/kg of titrate acidity and pH = 5.1.

The bromatological characterization of bee pollen from Argentina was made by Coronel et al. [14]. The moisture content ranged from 3.23 and 9.23%; the fat content varied from 1.76 to 6.76%; the proteins varied from 17.26 to 30.86%; the crude fibre varied from 0.1 to 2.46%; the ashes varied from 0.96 to 6.70%; the sugars constituted the major portion with values going up to 60%.

Vit and Santiago [25] evaluated the chemical composition of bee pollen in the fresh state collected from the Misintá páramo situated in the Venezuelan Andes. Their results showed that yellow pollen was the most frequent fraction, with 2.18% ash, 5.37% ether extract, 14.88% moisture and 37.32% proteins.

Almeida-Muradian et al. [2] studied the chemical composition of ten different pollens from south Brazil that included 17 botanical species, but the most significant were *Arecaceae*, *Asteraceae* and *Myrtaceae*. On average the moisture content was 7.4%, the protein was 20% dry basis, lipids 6% and 2.2% ash.

Human and Nicolson [17] evaluated the nutritional content of fresh, bee-collected and stored South African pollen from a single species (*Aloe greatheadii*). Their work revealed that the three types of pollen studied were significantly different, and that when the bees gathered and stored them, the moisture and carbohydrates contents increased while crude protein and lipids contents diminished.

2.2. Sugars

The analysis of sugars in five samples of bee pollen from different countries (Israel, China, Romania, and Spain:2) was assessed by Qian et al. [20] by means of high-performance thin layer chromatography (HPTLC), high-performance ligand exchange chromatography with pulsed amperometric detection (HPLC-PAD) and HPLC coupled to liquid chromatography–electrospray ionisation mass spectrometry (LC–ESI-MS). The main sugars in the pollen samples were glucose, fructose and sucrose, and in smaller amounts a trisaccharide was also detected. The amounts of glucose varied from 8.2% to 13.1%, fructose from 15.9% to 19.9% and sucrose from 14.8% to 18.4%.

2.3. Proteins and amino acids

Szczęśna [23] studied the composition in proteins and amino acids in unifloral pollens collected by honey bees from sixteen botanical origins (*Onagraceae*, *Caryophyllaceae*, *Artemisia*, *Agrimonia*, *Rheum*, *Cornus*, *Fragaria*, *Syringa*, *Ranunculus*, *Majorana* type, *Brassica*, *Sinapis alba*, *Sinapis arvensis*, *Campanula patula*, *Chelidonium maius*, *Polygonum bistort*). Their results showed that although the content of amino acids and proteins was dependent on the floral origin, contrarily the concentration of essential amino acids was fairly stable and hence not varying according to the botanical origin of the pollen. Furthermore, the proteins include the twenty-two basic amino acids, and are suitable for human consumption.

Human and Nicolson [17] reported that in pollen samples from South Africa at least 18 amino acids were present in the pollen, including the 10 essential amino acids, and that the ratios of essential to non-essential amino acids were not significantly different between the three types of pollen studied.

2.4. Fatty acids

According to Human and Nicolson [17] who studied fresh, bee-collected and stored South African pollens, 17 fatty acids were found on the bee collected and bee stored pollens while the fresh pollen had 13. The three prevailing fatty acids present in the fresh pollen were palmitic (24%), stearic (17%) and oleic (23%) acids. In the bee collected and bee stored pollens the main fatty acids were galoleic (42% and 24%, respectively), palmitic (14% and 22%, respectively) and oleic acids (12% and 13%, respectively).

Feás et al. [3] studied twenty two samples of organic bee pollen harvested in the Douro International Natural Park (Portugal). As to the fatty acid profiles, the main fatty

acid found was linolenic acid (41.52%), followed by linoleic (18.96%), palmitic (13.73%) and oleic (11.88%) acids.

Avni et al. [12] studied the nutritional aspects of honeybee-collected pollen from seven locations in Israel over an year, in terms of total protein, total fatty acids, and fatty acid composition. The overall mean pollen protein content was 39.8% and total fatty acids content was 3.8%. The overall means were 190 g for palmitic acid, 152 g for linoleic acid and 234 g for linolenic acid.

Čeksterytė et al. [13] studied the fatty acid composition in bee pollens. Twenty-two fatty acids were identified, including five Omega-3, four Omega-6 and three Omega-9 polyunsaturated fatty acids. Furthermore, the ratio of Omega-6 to Omega-3 fatty acids increased in beebread if the content of pollen increased.

Szczęśna [24] investigated the long-chain fatty acids composition of honeybee-collected pollen from selected countries: Poland, South Korea and China. By means of gaseous chromatographic analyses (CG-FID) they identified the some long-chain fatty acids: myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), -linolenic (C18:3), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0). In most of the studied samples the predominating acids were: linolenic acid (43%), followed by palmitic acid (28%) and linoleic acid (14%). As a consequence, the authors state that, due to a high concentration of essential fatty acids, pollen can be applied as a dietary supplement.

2.5. Phenolic compounds

Morais et al. [7] evaluated the phenolic content of pollens from five Portuguese Natural Parks. These mixed pollens were original from eight floral species: *Rosaceae*, *Cistaceae*, *Boraginaceae*, *Asteraceae*, *Fagaceae*, *Ericaceae*, *Myrtaceae* and *Fabaceae*. The phenolic compounds content varied between 10.5 and 16.8 mg GAE/g.

Feás et al. [3] reported phenolic contents varying from 12.9 to 19.8 mg GAE/g of extract and flavonoid contents varying from 4.5 to 7.1 mg of catechin equivalents/g in 22 samples of organic bee pollen harvested in the Douro International Natural Park (Portugal).

Pascoal et al. [1] determined the phenolic content of eight samples of bee pollen commercialized in Portugal, and which were bought from the market, being mostly original from Portugal and Spain. The total phenolic content varied from 18.55 to 32.15 mg/g, and the flavonoids varied between 3.92 and 10.14 mg/g.

The polyphenol composition of six different samples of bee pollen from Texas was determined by LeBlanc et al. [18]. Total polyphenols of pollen extracts ranged from 15.91 to 34.85 expressed as gallic acid equivalents (GAE) per mg/g pollen. The contents of flavones and flavonols varied between 2.66 and 5.48 expressed as quercetin equivalents (QE) per mg/g pollen. Flavonones contents ranged from 19.94 to 27.48 expressed as naringenin equivalents (NE) per mg/g pollen.

Šarić et al. [21] studied the phenolic composition of Croatian bee pollen by high performance liquid chromatography (HPLC) analysis. Seven phenolic compounds were detected belonging to flavonol (pinocembrin), flavanols (quercetin, kaempferol, galangin, and isorhamnetin), flavones (chrysin) and phenylpropanoids (caffeic acid).

Fanali et al. [16] used nano-liquid chromatography for the determination of polyphenols in bee pollen, and identified nine polyphenols, namely *o*-cumaric acid, *p*-coumaric acid, ferulic acid, myricetin, cinnamic acid, quercetin, naringenin, hesperetin and kaempferol.

Di Paola-Naranjo et al. [15] evaluated the anthocyanin composition of five samples of dark blue pollen from the species *Echium plantagineum* collected in Spain, by HPLC with diode array and mass spectrum detection. In their work they identified nine different anthocyanins, being the most abundant the petunidin-3-O-rutinoside, and present in successively lower amounts were delphinidin-3-O-rutinoside, delphinidin-3-O-glucoside, petunidin-3-O-glucoside, cyanidin-3-O-rutinoside, malvidin-3-O-rutinoside, cyanidin-3-(6''-malonylglucoside), cyanidin-3-O-glucoside and peonidin-3-O-rutinoside. The total anthocyanin content was found to vary from 45 to 80 mg/100 g, and the authors believe that differences may be due to the geographical origin of the pollens evaluated. Because of the high amount of anthocyanins present, they suggest that blue pollen could represent a significant source of phytochemicals.

2.6. Vitamins

The B complex vitamins were evaluated by de Arruda et al. [10] in seven fresh and dry commercial mixed bee pollen samples. In the fresh pollens, vitamin B₁ (thiamine) was found in concentrations ranging from 0.59 to 1.09 mg/100 g db; vitamin B₂ (riboflavin) from 1.73 to 2.56 mg/100 g db; vitamin B₃ or PP (niacin) from 6.43 to 15.34 mg/100 g db; vitamin B₆ (pyridoxin) from 0.33 to 0.79 mg/100 g db. They verified that these concentrations were not significantly altered by the dehydration process. The samples were then stored under different conditions: at room temperature

(with and without light) and frozen. After 1 year of storage, the concentration of vitamin B₁ stayed constant, while in the case of the other vitamins, the losses were variable according to the duration and not so much according to storage conditions.

A similar study was conducted by de Arruda et al. [11] on seven dried bee pollen samples collected from São Paulo, Brazil. In their study they evaluated the concentration of B complex vitamins and its vitamers by HPLC, with fluorescence detection. The concentrations (all expressed in dry basis) found for vitamin B₁ varied from 0.64 to 1.01 mg/100 g; for vitamin B2 from 1.77 to 2.56 mg/100 g; for vitamin PP from 7.27 to 14.43 mg/100 g; and for vitamin B6 from 0.33 to 0.77 mg/100 g.

Almeida-Muradian et al. [2] studied the chemical composition of ten different pollens from south Brazil that included 17 botanical species, but the most significant were *Arecaceae*, *Asteraceae* and *Myrtaceae*.. They verified the lack of vitamin C and beta-carotene but the occurrence of total carotenoids.

2.7. Miscellaneous

The analysis of coenzyme Q₁₀ in bee pollen using online cleanup by accelerated solvent extraction and high performance liquid chromatography has been done by Xue et al. [26] in twenty bee pollen products. Their results showed contents varying from not detectable to 192.8 mg/kg.

Silva et al. [22] evaluated the chemical composition of bee pollen from Brazil. The mass spectroscopy (MS), ultraviolet (UV), melting point (mp) and Nuclear Magnetic Resonance (NMR) spectra were the methods used to identify the structures of compounds isolated from the pollen: β -sitosterol, tricetin, selagin, 8-methoxyherbacetin, d-mannitol, d-mannitol hexaacetate, naringenin, and isorhamnetin.

3. EFFECTS OF BEE POLLEN ON HUMAN HEALTH

Bee pollen can be seen as a healthy foodstuff with an extensive variety of therapeutic properties. Feás et al. [3] summarizes some of the therapeutic properties of bee pollen that were already confirmed by modern science: antifungal, antimicrobial, antioxidant, anti-radiation, hepatoprotective, chemopreventive, anticancer and anti-inflammatory activities. In addition, it has been reported to trigger beneficial effects in the prevention of prostate problems, arteriosclerosis, gastroenteritis, respiratory diseases, allergy desensitization, improving the cardiovascular and digestive systems, body immunity and delaying aging [1,6,27]. Table 2 shows some of the published

works that evaluated the effects of bee pollen on different biological activities. According to Almeida-Muradian et al. [2] these therapeutic and protective effects have been related to the presence of polyphenols.

Table 2. Documented bioactivities of bee pollen.

	Antioxidant activity	Anti-inflammatory activity	Antimicrobial activity	Antifungal activity	Allergy	Liver protection	Antimutagenic and anticancer activities	Toxicity protection
Almaraz-Abarca et al. [28]	•							
Bevzo and Grygor'eva [29]						•		
Carpes et al. [30]			•					
Chantarudee et al. [31]	•							
Eraslan et al. [32]						•		
Eraslan et al. [33]								•
Feás et al. [3]	•	•	•					
Furusawa et al. [34]							•	
LeBlanc et al. [18]	•							
Mărghitaș et al. [35]	•							
Maruyama et al. [36]		•						
Medeiros et al. [4]					•			
Morais et al. [7]	•		•					
Ozcan [37]				•				
Pascoal et al. [1]	•	•	•				•	
Pinto et al. [38]							•	
Šarić et al. [21]						•	•	
Silva et al. [22]	•							
Yıldız et al. [39]						•		

3.1. Antioxidant activity

Pascoal et al. [1] evaluated the antioxidant activity of eight commercial bee pollens by two methods: inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) and scavenging of DPPH (radical 2,2-diphenyl-1-picrylhydrazyl). The antioxidant activity varied from 0.35 to 3.70 mg/mg as given by the first method, while by the DPPH it was higher and varied from 2.98 to 6.69 mg/mg.

The free radical scavenging capacity of pollens from five Portuguese Natural Parks were evaluated by Morais et al. [7] according to two methodologies: DPPH method and β -carotene bleaching (BCB) assay. The results obtained with the DPPH method varied from 2.16 to 5.87 mg/mL, whereas in the β -carotene bleaching assay varied from 3.11 to 6.52 mg/mL.

Feás et al. [3] studied the antioxidant effect of twenty two samples of organic bee pollen from Portugal. The scavenger activity and β -carotene bleaching assays values were on average 3.0 mg/mL and 4.6 mg/mL mg/mL, respectively, thus being similar to those found by Morais et al. [7].

Almaraz-Abarca et al. [28] investigated the variation of antioxidant activity between honeybee-collected pollens from different botanical origins. The antioxidant activities of total extracts of a mixture of honeybee-collected pollen and its six constituent pollens were determined by two methodologies: lipid peroxidation assay by the TBARS test and DPPH method. The results revealed that all extracts presented antioxidant properties acting as radical scavengers and also as inhibitors of lipid peroxidation. Furthermore, the antioxidant activities were variable among species but were not evidently associated to the contents of flavonols in the pollen.

The *in vitro* antioxidant capacity of twelve honeybee-collected pollens of selected floral origin harvested from Romania was evaluated by Mărghițaș et al. [35]. The antioxidant activities were assessed by three different methods: DPPH assay, Trolox equivalent antioxidant capacity procedure and ferric ion reducing antioxidant power assay (FRAP). Their results indicated that the antioxidant activities varied according to each floral species and were not noticeably related to the contents in phenolic compounds.

Silva et al. [22] evaluated the free radical scavenging activity of different solvent extracts from Brazilian bee pollen by using the DPPH assay. Their results showed that the antioxidant activity was greater for the ethyl acetate extract, followed by the ethanol

and finally the hexane extracts, hence suggesting that the ethyl acetate extract, rich in flavonoids, is a good scavenger of active oxygen species.

The antioxidant activity of six different samples of Sonoran Desert bee pollen were studied by LeBlanc et al. [18] using the DPPH and FRAP assays. The extracts were produced using eight different solvents: water, methanol, ethanol, propanol, 2-propanol, acetone, dimethylformamide and acetonitrile. The antioxidant activity measured by the DPPH assay varied from 0.90% (for terpenine bush pollen and acetonitrile solvent) to 89.05% (for mimosa pollen and dimethylformamide solvent). The FRAP assay yield much lower values, varying from 0.05% (for palm pollen and propanol solvent) to 4.15 (for mimosa pollen and dimethylformamide solvent).

The work by Chantarudee et al. [31] compared the free radical scavenging activity of corn pollen collected from *Apis mellifera* hives with floral corn pollen collected at corn fields in Nan province, Thailand. The extraction solvents used were methanol, dichloromethane (DCM) and hexane, and the extracts were analysed by the DPPH assay, evaluating the percentage scavenging activity and the effective concentration at 50% (EC_{50}). From the three solvents studied, the DCM exhibited the highest antioxidant activity. The work allowed concluding that the antioxidant activity of the bee pollen derived from corn was higher (for DCM: $EC_{50} = 428.6 \pm 29.0 \mu\text{g/ml}$) when compared to that of the floral corn pollen (for DCM: $EC_{50} = 365.2 \pm 38.8 \mu\text{g/ml}$).

3.2. Anti-inflammatory activity

Pascoal et al. [1] evaluated eight bee pollens at sale in the market in Portugal in relation to their anti-inflammatory activity, assessed using the hyaluronidase enzyme. They found that all samples evidenced anti-inflammatory activity, with percentages of inhibition varying from 12.20% to 25.17%.

The anti-inflammatory effect of bee pollen ethanol extract from Spanish origin on carrageenan-induced rat hind paw edema, as well as the mechanism of anti-inflammatory action was investigated by Maruyama et al. [36]. Their results indicated that the bee pollen showed a potent anti-inflammatory capacity and also that some flavonoids that were present in the bee pollen may partially contribute for some anti-inflammatory effects. Hence they concluded that bee pollen could be helpful both as a dietary supplement and also as a functional food.

Feás et al. [3] studied the anti-inflammatory activity of twenty two samples of organic bee pollen from Portugal, assessing the anti-inflammatory activity using the hyaluronidase enzyme, and found it varying from 12.20% to 25.17%.

3.3. Antimicrobial activity

Morais et al. [7] studied the antimicrobial activity of pollens from five Portuguese Natural Parks, against eight test microorganisms (*Salmonella typhi* ESA 101, *Staphylococcus aureus* ESA 72, *Bacillus cereus* ESA 55, *Escherichia coli* ESA 15, *Zygosacharomyces mellis* ESA 35, *Zygosacharomyces bailii* ESA 1307, *Zygosacharomyces rouxii* ESA 23, *Candida magnolia* ESA 11). Their results showed that all the extracts evidenced antimicrobial activity, and showed different selectivity and different Minimum Inhibitory Concentration for each microorganism. They also observed that the occurrence of pollen and the type of pollen affected the growth of the microorganisms under study (Gram-positive bacteria, Gram-negative bacteria, yeasts) in different ways.

Feás et al. [3] studied the antimicrobial activity of twenty two samples of organic bee pollen from Portugal against seven microorganisms (*S. aureus* ATCC 6538, *S. aureus* ESA 159, *P. aeruginosa* ATC, *P. aeruginosa* ESA 22, *E. coli* ATCC, *E. coli* ESA 37, *C. glabrata* ATC). They observed that the antimicrobial activity varied significantly with the pollen sample, for all the strains under study. The most sensitive microorganisms were both strains of *S. aureus*, one of the most representative bacteria involved in respiratory diseases. These showed minimum inhibitory concentrations ranging from 1.81 and 4.28 mg/mL.

The antimicrobial activity of eight commercial bee pollens was evaluated by Pascoal et al. [1] against seven microorganisms (*S. aureus* ATCC 6538, *S. aureus* ESA 159, *P. aeruginosa* ATC, *P. aeruginosa* ESA 22, *E. coli* ATCC, *E. coli* ESA 37, *C. glabrata* ATC). Their results showed that all the samples exhibited antimicrobial actions, with *Staphylococcus aureus* revealing as the most sensitive and *Candida glabrata* as the most resistant among the microorganisms studied.

Carpes et al. [30] characterized the antimicrobial potential of thirty six pollen extracts from south Brazil using nine microorganisms: *Bacillus subtilis* ATCC 21.332, *Pseudomonas aeruginosa* ATCC 15.442, *Streptococcus mutans* Ingbritt 1600, *Staphylococcus aureus* ATCC 25.923, *Klebsiella pneumoniae*, *Agrobacterium tumefaciens*, *Xanthomonas vesicatoria*, *Xanthomonas axonopodis* and *Pseudomonas*

syringae). Their results showed that, although the pollen extracts presented high concentrations of phenolic compounds and flavonoids, they were not capable of inhibiting the microorganism growth.

3.4. Antifungal activity

The inhibitory effects of pollen extracts from different regions of Turkey on mycelial growth of *Aspergillus parasiticus* strain NRRL 2998 were investigated *in vitro* by Ozcan [37], who found that the less active concentration headed for the fungus in the experiments was 2% of the pollen extract.

3.5. Allergy

The bee pollen phenolic extract (BPPE) contains phenolic compounds, and specially flavonoids. Since some flavonoids have shown immunomodulatory activity, Medeiros et al. [4] evaluated the effect of BPPE and the flavonoid myricetin (one of the compounds present) in the murine model of ovalbumin-induced allergy. The BPPE treatment in dosages of 200 mg/kg showed inhibition of the paw edema, and the treatment with myricetin at 5 mg/kg also inhibited pulmonary cell migration and IgE and IgG1 OVA-specific production. Their results indicate that myricetin is one flavonoid that proved to have an anti-allergic effect and therefore constitutes a possible instrument to treat allergies.

3.6. Liver protection

Eraslan et al. [32] investigated the *in vivo* effect of carbaryl, which is a pesticide, on some biochemical changes in rats, namely the ameliorative effect of bee pollen. The results obtained allowed verifying that carbaryl originated negative changes in the majority of the markers of oxidative stress and also in the biochemical parameters investigated in the serum. However, these effects were alleviated when a water-solubilized extract of bee pollen was administrated in dosages of 50 and 100 mg/kg bw/day.

Šarić et al. [21] investigated the *in vivo* oxidant/antioxidant effects of flavonoid from Croatian bee pollen. The investigators found that the administration of bee pollen as a food supplement in a concentration of 100 mg/kg bw modulated antioxidant enzymes in the mice liver, brain and lysate of erythrocytes and reduced hepatic lipid peroxidation.

The hepatoprotective potential of bee pollen was assessed by Yıldız et al. [39], who evaluated the effect of chestnut bee pollen on hepatic damages induced in rats. The results obtained from their work demonstrated that the chestnut bee pollen could effectively protect the hepatocytes from the oxidative stress, thus promoting the healing of the induced liver damage.

Bevzo and Grygor'eva [29] studied the effect of bee pollen extract on glutathione system activity in mice liver under X-ray irradiation. Their results allowed establishing that small X-irradiation doses activate the lipid peroxidation and antioxidant system enzymes in the liver of the mice. However, the administration of bee pollen extract to the animals normalized the activity of several glutathione system enzymes in the mice liver.

3.8. Antimutagenic and anticancer activities

Pascoal et al. [1] studied eight commercial bee pollens in relation to their antimutagenic activity. The anti-genotoxic effects of bee pollen on *S. cerevisiae* were tested up to 0.75 mg/mL in a 0.5% fixed ethyl methanesulfonate concentration, which produces random mutations in genetic material by nucleotide substitution. Their results showed that all the samples demonstrated antimutagenic activity, although some of them showed to be more effective in diminishing the number of gene conversion colonies and mutant colonies than others.

The genotoxicity/antigenotoxicity of bee pollen from *Cystus incanus* and *Salix alba* and their derivative extracts in yeast and human cells (lymphocytes) was investigated by Pinto et al. [38]. They found that the pollens were not genotoxic and were capable of reducing the damage in the chromosomes produced by the three cancer drugs used. Hence they concluded that the bee pollens could be used as a safe food supplement with chemoprotective/chemopreventive properties.

Šarić et al. [21] investigated the *in vivo* effects of flavonoid from Croatian bee pollen in terms of estrogenic/anti-estrogenic activity and gene expression profile. The investigators found that the administration of bee pollen as a food supplement in a concentration of 100 mg/kg bw induced 25% of anti-estrogenic properties and on the other hand no estrogenic activity was found. Differential gene expression profile analyses after bee pollen enriched diet identify underexpressed gene Hspa9a, Tnfsf6 (liver) and down-regulated gene expression of Casp 1 and Cc121c (brain) which are important in the apoptosis pathway and chemotaxis. In face of these results, the authors

postulated that bee pollen possess a noticeable source of compounds with health protective potential.

Furusawa et al. [34] studied the antitumour potential of the water-soluble fraction (Cernitin T60) of pollen extract on Lewis lung carcinoma implanted intraperitoneally in syngeneic mice. Cernitin T60 was not cytotoxic in cell cultures at concentrations up to 2.5 mg/ml and at concentrations of 0.5 g/kg it meaningfully extended the lifetime of mice having the tumour without any detectable adverse effects. Furthermore, Cernitin T60 revealed beneficial synergetic therapeutic effects on the lifetime of mice when it was combined with standard cytotoxic antitumor drugs. The authors concluded that Cernitin T60 seems to be a powerful immunostimulator of macrophages.

3.9. Toxicity protection

The protecting capacity of bee pollen against propoxur toxicity in an *in vivo* model was evaluated by Eraslan et al. [33]. The rats were administrated bee pollen in dosages of 100 mg/kg bw/day and the effect of pollen administration on the propoxur toxicity was evaluated after 14 days in blood and tissues (liver, kidney, brain and heart) collected from the animals, by means of oxidative stress markers and some other biochemical parameters. The data obtained allowed verifying that propoxur caused undesirable changes in the majority of the biochemical parameters investigated and the administration of bee pollen alleviated these effects.

3. CONCLUSION

The evolution as to know the composition of pollen has been important in the past years, not only at the physico-chemical level, but also from the nutritional and health points of view, by searching for bioactive compounds with biological effects on the human body.

The information found on the scientific literature reveals an increasing interest in developing studies, both *in vivo* and *in vitro* to explore the true effects of bee pollen on human health and well-being. Among the most frequently investigated effects of bee pollen stand its antimicrobial, anti-inflammatory, antioxidant and antimutagenic activities.

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