applied sciences

MDPI

Article

Chemical Characterization and Antioxidant Activity of Apilarnil, Royal Jelly, and Propolis Collected in Banat Region, Romania

Dragoș Moraru [1,†], Ersilia Alexa [2], Ileana Cocan [2,*], Diana Obiștioiu [3], Isidora Radulov [3], Eliza Simiz [1], Adina Berbecea [3], Adrian Grozea [1], Monica Dragomirescu [1], Teodor Vintilă [1] and Silvia Pătruică [1,†]

[1] Faculty of Bioengineering of Animal Resources, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului No. 119, 300645 Timisoara, Romania; dragos.moraru@usvt.ro (D.M.); elizasimiz@usvt.ro (E.S.); adriangrozea@usvt.ro (A.G.); monicadragomirescu@usvt.ro (M.D.); teodorvintila@usvt.ro (T.V.); silviapatruica@usvt.ro (S.P.)
[2] Faculty of Food Engineering, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului No. 119, 300645 Timisoara, Romania; ersiliaalexa@usvt.ro
[3] Faculty of Agriculture, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului No. 119, 300645 Timisoara, Romania; dianaobistioiu@usvt.ro (D.O.); isidora_radulov@usvt.ro (I.R.); adina_berbecea@usvt.ro (A.B.)
* Correspondence: ileanacocan@usvt.ro
† These authors contributed equally to this work.

Abstract: Bee products have a millennia-old tradition in human apitherapy, thanks to their numerous curative properties. The aim of this study was to perform a physicochemical characterization, including the percentage of water content, dry matter, pH, acidity, impurity content, protein, ash, and macro and micro minerals, and to evaluate the antioxidant activity of apiculture products, including apilarnil, royal jelly, and propolis. Samples were collected from our apiary in the Banat region of Romania in 2022 as well as from the market. Apilarnil and royal jelly were analyzed in pure and freeze-dried form, and propolis in pure and tincture form. Our results show that there is a large variability in the chemical composition and antioxidant activity of the three bee products studied, depending on the form of presentation and source of origin. In the case of commercial bee products, the best results were obtained in terms of chemical composition, including the content of macro and micro nutrients. All the samples analyzed showed acidity, pH, and impurity content values within the limits allowed by the quality standards. Apilarnil from our own apiary recorded the highest values for antioxidant activity, followed by propolis tincture and fresh propolis from own apiary.

Keywords: apilarnil; royal jelly; propolis; physicochemical composition; antioxidant activity

check for updates

Citation: Moraru, D.; Alexa, E.; Cocan, I.; Obiștioiu, D.; Radulov, I.; Simiz, E.; Berbecea, A.; Grozea, A.; Dragomirescu, M.; Vintilă, T.; et al. Chemical Characterization and Antioxidant Activity of Apilarnil, Royal Jelly, and Propolis Collected in Banat Region, Romania. *Appl. Sci.* **2024**, *14*, 1242. https://doi.org/10.3390/app14031242

Academic Editors: Monica Gallo and Anna Lante

Received: 2 January 2024
Revised: 26 January 2024
Accepted: 30 January 2024
Published: 2 February 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Bee products contain biologically active principles with an antioxidant role [1–3]. The biological activities of active products can vary widely and are influenced by a number of factors, including the source of the flowers, the location of the apiary, the experience of the beekeeper, the environmental conditions, and the breed of bee [1]. The variable and complex composition of bee products, as well as the variety of laboratory methods used to study them, explain the wide range of reported results on the antioxidant activity of bee products [4].

Apilarnil is a product obtained by centrifuging drone larvae harvested at the age of 7 days together with the entire contents of the cells in which they develop. After harvesting it is mixed and becomes a fine-grained viscous substance [5]. The high content of biologically active substances in apilarnil determines many pharmacological characteristics, especially the antioxidant, immunotropic, adaptogenic, and anabolic actions [6]. The studies carried out by Bogdanov [7] showed that drone larvae contain estradiol, prolactin, progesterone, and testosterone, showing both estrogenic and androgenic effects.

Royal jelly is a yellowish-white bee product that is produced by the hypopharyngeal and mandibular glands. It has a sour taste and a viscous, gelatinous consistency, with a slight smell similar to phenol [8–13]. It is produced by nurse bees, aged between 5 and 15 days [11], being used within the bee family to feed queens and larvae. Its secretion is correlated with the abundance of nectar and pollen collection [14]. Royal jelly has an acidic pH ranging from 3.6 to 4.2. It is primarily composed of water (60–70%), with additional components including proteins (12%), lipids (5–6%), and small amounts of vitamins and mineral salts. Additionally, it contains a considerable number of amino acids [8].

Propolis is a substance produced by honey bees using resinous materials collected from different parts of plants, including leaves, flowers, and buds. The bees add glandular secretions, residues from wax digestion, and pollen to the mixture. The resulting product has various uses and benefits [8,15]. The raw material for its production is harvested mainly from plants such as willow, conifers, beech, poplar, oak, alder, ash, chestnut, and others [16–20]. Each year, about 100–300 g of propolis can be obtained from a family of bees from the cleaning of the interior of the hives and the frames during the active season [14]. It consists of approximately 50% resins, 30% wax, 10% various essential oils, 5% pollen, and 5% various organic compounds including polyphenols, amino acids, minerals, ethanol, vitamin A, vitamin E complex, and vitamin B [8,21,22]. Propolis is an antimicrobial agent used against bacteria, viruses, and other pathogenic micro-organisms [23–27] and has more than 70 pharmaceutical properties. Research was carried out on the activity of propolis and it was found that it can be used as an additive with an antimicrobial, immuno-modulatory, antioxidant, antiinflammatory, antiparasitic role [28,29]. Propolis can be added to food, drinks, and cosmetics. It is available commercially in various forms such as capsules, creams, throat lozenges, and mouthwash solutions [30].

The main objectives of this study were to evaluate the chemical composition of some bee products collected directly from the apiary (apilarnil, royal jelly, and propolis) compared with the same raw products purchased commercially as commercial lyophilized products (apilarnil and royal jelly) and as tincture (propolis). At the same time, we evaluated the antioxidant activity of those bee products.

2. Materials and Methods

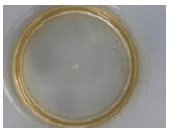
2.1. Samples of Bee Products

We analyzed the apilarnil, royal jelly, and propolis products (Table 1) obtained in May 2022. The analyzed propolis came from our own apiary and from the market, the form of presentation being raw propolis and propolis tincture prepared by macerating 20 g of propolis (from our own apiary) in 100 mL of ethyl alcohol 96%. Royal jelly was tested in freeze-dried and pure form from commercial and freshly harvested origins. Apilarnil was subjected to research in the same forms as royal jelly but the lyophilized apilarnil was obtained by lyophilizing commercial pure apilarnil using the Unicryo MC4L −60 °C lyophilizer (Uniequip, Planegg, Germany). The apiary chosen for collecting samples in fresh form is located in the town of Caransebeș, Banat region, Romania (45.024′48.6″ N 22.012′53.7″ E). The samples were stored in glass containers at a temperature of 0 ± 5 °C in a refrigerator.

Table 1. Bee product samples analyzed.

No. Sample	Product Type	Source of Origin	Abbreviation	Photo
1	Apilarnil pure	Own apiary	AS	

Table 1. *Cont.*

No. Sample	Product Type	Source of Origin	Abbreviation	Photo
2	Lyophilized apilarnil	Market	ALC	
3	Apilarnil pure	Market	AP	
4	Royal jelly pure	Own apiary	LMS	
5	Lyophilized royal jelly	Market	LML	
6	Royal jelly pure	Market	LMC	
7	Propolis raw	Own apiary	PS	
8	Propolis raw	Market	PC	
9	Propolis tincture	Own apiary	PT	

2.2. Determination of Water Content

Water content was determined according to the SR 784-3:2009 [31] standard by the oven drying method. Two determinations were performed for each sample. From each sample, with the exception of commercial (freeze-dried) royal jelly, 3 g was weighed. From the sample of royal jelly, 1.5 g was weighed. The weighed samples were placed in an oven (BINDER GmbH, Tuttlingen, Germany) at 103 ± 2 °C until they reached constant mass. The dehydrated samples were removed from the oven, placed in the exicator where they were allowed to cool, and then weighed. The result was calculated using Equation (1):

$$\text{Water content} = [(G1 - G2)/(G1 - G3)] \times 100\ (\%) \quad (1)$$

where G1 is the weight of the container and sample before drying (g), G2 is the weight of the container and sample after drying (g), and G3 is the weight of the empty container (g).

2.3. Determination of Dry Matter

Two determinations were performed for each sample. The determination was made according to the formula:

$$\text{Dry matter} = 100 - \text{Water content}\ (\%)$$

2.4. Determination of Acidity

The standardized method SR 784-3:2009 [31] was used to determine acidity. Two determinations were performed for each sample. Thus, 1 g was weighed from each sample of the apiculture products and 5 mL of demineralized water was added. The samples thus prepared were homogenized in a DLAB shaker, SK-L330-PRO (Qingdao, China) for 30 min and then filtered through Whatman filter paper 0.45 µm, (Sigma-Aldrich, Taufkirchen, Germany; Merck KGaA, Darmstadt, Germany). The filtered samples were titrated with 0.1 n sodium hydroxide solution (p.a. Sigma-Aldrich, Taufkirchen, Germany) in the presence of 1% phenolphthalein (alcoholic solution) until a pink color appeared and persisted for 30 s [32,33]. The room temperature in which the acidity determination was carried out was between 23–24 °C, and Equation (2) was used to calculate and express the results:

$$\text{Acidity} = [(V \times 0.1)/10] \times 100 \text{ (mL NaOH 0.1 n/100 g bee product)} \quad (2)$$

where V represents the volume of sodium hydroxide solution used in the titration (mL), and 0.1 represents the normality of sodium hydroxide solution used for titration.

2.5. Determination of pH

The inoLab pH 720 pH meter (Xylem Analytics, Weilheim, Germany) was used to determine the pH, and the amount of bee product used was 1 g/sample. Two determinations were performed for each sample. They were dissolved in 30 mL of water and homogenized using the DLAB shaker (SK-L330-PRO, China) for 30 min [33,34]. The pH of the test samples was determined at a room temperature of 23–24 °C and the pH working range was -2000 ± 19.999 with an accuracy of ± 0.005.

2.6. Determination of Impurities

The experiment was conducted following the SR 784-3:2009 standard [31] with minor changes. From the propolis samples, 1 g was weighed and dissolved in 10 mL of 96% alcohol. From the samples of royal jelly and apilarnil, 1 g was weighed and dissolved in 10 mL of water. The mixture was homogenized for 30 min using the DLAB shaker (SK-L330-PRO, China). Afterwards, the filter papers were prepared and weighed, and after weighing, the solutions were filtered. The filtered samples were dried in an oven at 103 °C for 10 min to remove moisture from the filter paper before being weighed. Two determinations were performed for each sample. The percentage of impurities was calculated using Equation (3):

$$I = \left(\frac{m_2}{m_1}\right) \cdot 100 \ (\%) \quad (3)$$

where I represents the quantity of impurities (%), m_1 represents the mass of the sample taken for analysis (g), and m_2 represents the mass of residue left on the filter paper after drying (g).

2.7. Determination of Protein

The protein content of apilarnil and royal jelly samples was determined by dissolving 0.5 g of each sample in 25 mL of distilled water. The resulting solutions were filtered using glass tubes and filter paper. The protein content was then measured using the Lowry method, with bovine serum albumin (BSA) as the standard and Folin–Ciocalteu's phenol reagent as the reagent [35,36]. The color reaction was carried out using the clear solutions of apilarnil and royal jelly obtained previously, to which were added alkaline solution (containing NaOH, Na_2CO_3, sodium potassium tartrate, and $CuSO_4$) and Folin–Ciocalteu reagent. The extinction was measured using a T 60U spectrometer (PG Instruments Ltd., Lutterworth, UK) at 660 nm against the blank solution. Two determinations were performed for each sample. The protein content was determined using the calibration line equation and calculated based on the Formula (4):

$$p = (Emed - Econtrol + 0.021) \times 10 \times 50/2.23455 \text{ (mg protein/g mL)} \quad (4)$$

where p represents the quantity of protein (mg protein/mL), Emed represents the average extinction of duplicate samples, 10 is the correction factor for expressing the protein content per 1 mL sample, and 50 is the correction factor for expressing the protein content per 1 g sample.

2.8. Determination of Ash

Ash content was determined according to the SR 784-3:2009 standard [31]. The empty crucibles required for each sample were kept at a temperature of 525 °C for two hours in the calcination furnace (190945, Nabertherm, Lilienthal, Germany), and later they were cooled and submitted to weighing. In the next step, 1 g samples of each bee product were weighed, placed in the cooled crucibles, and later the crucibles were reintroduced into the calcination furnace (190945, Nabertherm, Lilienthal, Germany) and were calcined up to a temperature of 525 °C. After the completion of calcination, the crucibles were cooled and weighed individually. Two determinations were performed for each sample.

The ash content is expressed as a percentage and determined according to Equation (5):

$$C_{ash} = (m - m_1)/(m_2 - m_1) \text{ (\%)} \quad (5)$$

where C_{ash} = total ash content, m is the mass of the crucible with ash after the calcination process, in grams, m_1 is the mass of the empty crucible, in grams, and m_2 is the mass of the crucible with the bee products before the calcination process, in grams.

2.9. Determination of Mineral Substance Content (Ash)

To determine the ash content in the calcined samples, 10 mL of hydrochloric acid was added to each sample. The resulting samples were then placed in glass test tubes to determine the content of microelements and macroelements. For each bee product, double determinations were made, resulting in 18 graduated flasks of 50 mL. The samples were filtered and filled with double-distilled water up to the 50 mL mark of each graduated flask. The atomic absorption spectrometry technique was used for the determinations, with a VARIAN 240 FS spectrophotometer (Palo Alto, CA, USA) and Centipur Merk multielement standard solution for calibration. The device was set to an air–acetylene ratio of 13.50:2 and a nebulizer absorption rate of 5 mL/min. Two determinations were performed for each sample. Conditions are presented in Table 2.

Table 2. Parameters used when reading mineral elements.

Metal	Wavelength λ (nm)	Lamp Current (mA)	Slit Width (nm)
Pb	217.0	10	1.0
Ca	422.7	10	0.5
Ni	232.0	4	0.2
Mg	285.2	4	0.5
Fe	248.3	5	0.2
Cu	324.8	4	0.5
Na	589.0	3	0.8
Cr	357.9	8	0.2
Zn	213.9	5	1.0
K	766.5	4	0.2
Mn	279.5	5	0.2
Cd	228.8	4	0.5

2.10. Determination of the Antioxidant Capacity via DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The DPPH test is a widely used classical method for determining the antioxidant capacity of various extracts. The analysis was conducted following the method outlined by Cadariu et al. [37], with minor modifications. For each sample, five alcoholic extracts of varying concentrations (0.5, 1, 2.5, 5, and 10 mg/mL) were prepared using 70% ethyl alcohol. In the present study, 0.5 mL of each alcoholic extract was taken and mixed with 2.5 mL of 0.3 mM DPPH solution in ethanol (Calbiochem®, EMD Millipore Corp., Billerica, MA, USA, lot: D00174004). The samples were left in the dark at room temperature for 30 min, and the absorbance was measured at 518 nm using a UV–Vis spectrophotometer (Analytic Jena Specord 205, Jena, Germany). A control sample was also prepared, replacing the extract with 70% ethyl alcohol. A positive control of ascorbic acid 0.16 mg/mL in 70% (*v*/*v*) ethanol was used. The ascorbic acid was purchased from Lach-Ner Company (Neratovice, Czech Republic). The radical scavenging activity (RSA) of the extract was calculated using Equation (6):

$$\text{RSA (\%)} = (\text{A control} - \text{A sample})/(\text{A control}) \times 100 \quad (6)$$

where RSA represents the radical scavenging activity of the extract in percent, A control represents the absorbance of the control sample, and A sample represents the absorbance of the sample measured at 518 nm. The antioxidant capacity of the extracts was measured according to their IC50 value and compared with that of ascorbic acid.

2.11. Statistical Analysis

The results were obtained through the use of IBM SPSS 22 statistical software. Statistical differences ($p < 0.05$) between the bee products analyzed were calculated using the Anova program with the Tukey test.

3. Results and Discussion

3.1. Chemical Composition

3.1.1. Chemical Composition of Apilarnil

Apilarnil is a product of yellowish color, milky consistency, and sour taste, obtained after triturating 7-day-old drone larvae collected together with the cell contents [38]. The composition of the apilarnil is variable and depends on the age of the larvae, the food sources to which the bees have access, and the beekeeping season.

The fresh apilarnic we analyzed had a water content of 68.54%, acidity 26.62 mg/g, and pH 5.74 (Table 3). The commercial samples had an impurity percentage of 5.47% and those harvested from the apiary 15.28%, with statistically significant differences ($p < 0.05$) between the two sources. Large variations in protein content were observed in the samples of fresh commercial apilarnil (22.04 mg/g) and those harvested from the apiary (44.86 mg/g). The ash content of the samples analyzed was 0.88%, with maximum values observed in the commercial samples (1.45 mg/g and 1.89 mg/g respectively). The main macroelement found was phosphorus 1233.00 mg, followed by potassium 787.65 mg, magnesium 369.93 mg, calcium 284.23 mg, and sodium 144.02 mg. The best represented microelement was iron 12.31 mg, followed by zinc 8.66 mg, chromium 3.29 mg, manganese 4.71 mg, and copper 3.71 mg.

Freeze-dried apilarnil had a water content of 6.78%; protein was represented at 117.25 mg/g and ash 1.89%. The weight of macro- and microelements was close to that observed in fresh apilarnil (Table 3).

Of the contaminants analyzed, nickel and lead were not detected in any sample of fresh or freeze-dried apilarnil, and cadmium was identified in the range of 1.34 ppm, with higher values in commercial samples (Table 3).

Table 3. Chemical composition of fresh and lyophilized apilarnil.

	Fresh Apilarnil		Lyophilized Apilarnil
	Own Apiary Mean ± SD	**Market** Mean ± SD	Mean ± SD
Water content (%)	68.54 ± 0.295 ^{a}	78.58 ± 0.005 ^{b}	6.78 ± 0.470 ^{B}
DM (%)	31.36 ± 0.274 ^{a}	21.43 ± 0.017 ^{b}	93.19 ± 0.475 ^{B}
Acidity	26.62 ± 0976 ^{a}	12.62 ± 0.69 ^{b}	78.25 ± 0.160 ^{B}
pH	5.74 ± 0.010 ^{a}	4.17 ± 0.152 ^{b}	5.55 ± 0.050 ^{A}
Impurities (%)	15.28 ± 0.091 ^{a}	5.47 ± 0.026 ^{b}	5.65 ± 0.735 ^{A}
Protein (mg/g)	44.86 ± 0.335 ^{a}	22.04 ± 1.235 ^{a}	117.25 ± 4.695 ^{B}
Ash (%)	0.88 ± 0.025 ^{a}	1.45 ± 0.010 ^{b}	1.89 ± 0.010 ^{B}
Macroelements			
Na	144.02 ± 52.962 ^{a}	159.53 ± 8.209 ^{a}	184.00 ± 8.174 ^{A}
Ca	284.23 ± 176.104 ^{a}	309.11 ± 36.465 ^{a}	413.81 ± 40.722 ^{A}
K	787.65 ± 780.596 ^{a}	578.89 ± 338.69 ^{a}	2252.91 ± 1262.09 ^{A}
Mg	369.93 ± 372.89 ^{a}	206.06 ± 11.377 ^{a}	459.60 ± 187.333 ^{A}
P	1233.00 ± 1256.88 ^{a}	375.78 ± 85.878 ^{a}	1343.25 ± 691.903 ^{A}
Microelements			
Fe	12.31 ± 9.461 ^{a}	7.86 ± 2.064 ^{a}	32.58 ± 19.219 ^{A}
Mn	4.71 ± 1.605 ^{a}	4.79 ± 0.919 ^{a}	4.22 ± 0.403 ^{A}
Cu	3.71 ± 1.767 ^{a}	4.54 ± 0.989 ^{a}	7.40 ± 3.478 ^{A}
Zn	8.66 ± 6.455 ^{a}	9.34 ± 2.743 ^{a}	30.10 ± 23.129 ^{A}
Cr	3.29 ± 0.848 ^{a}	6.80 ± 0.049 ^{b}	6.40 ± 0.127 ^{A}
Contaminants			
Ni	Undetectable	Undetectable	Undetectable
Cd	1.34 ± 0.268 ^{a}	2.22 ± 0.374 ^{a}	1.68 ± 0.516 ^{A}
Pb	Undetectable	Undetectable	Undetectable

Results are expressed as the two-determination mean ± standard deviation (SD). Different letters indicate statistically significant differences between samples in a row ($p < 0.05$) according to the Tukey test.

The results obtained fall within the range of data presented in the specialized literature; the differences may be the result of geographical and meteorological conditions, the beekeeping season in which the harvesting was completed, food sources, etc. The research carried out by Margaoan et al. [38] highlighted the following chemical composition for apilarnil: water 73.25%, total protein 9.47%, lipids 8.38%; while the royal jelly analyzed had a water content of 66.03%, total protein 11.14%, and lipids 3.96%.

Kim et al. [39] reported the nutritional profile of fresh apilarnil from Apis mellifera pupae drones aged 21–24 days as moisture 74.23 g/100 g, protein 11.05 g/100 g, fat 8.19 g/100 g, ash 0.85 g/100 g, and for dry apilarnil obtained from drone pupae aged 16–20 days [40] moisture 1.69 g/100 g, protein 48.52 g/100 g, fat 23.41 g/100 g, and ash 4.05 g/100 g. The protein level of bee brood (pupae and larvae) evaluated by Choi et al. [41] was 46.4–46.73 g/100 g and the fat level 18.84–20.75 g/100 g.

3.1.2. Chemical Composition of Royal Jelly

Royal jelly is a valuable source of nutrients and bioactive components whose composition depends on a multitude of factors such as the beekeeping season, climatic conditions, the ecosystem in which the bees live, honey sources to which they have access, pollution

sources within flight of bees, and also the genetics of the colony. In the hive, royal jelly intervenes in the phenotypic development of worker bee larvae when they benefit from abundant feeding over a longer period of time, turning them into queens, and it also plays an important role in the social behavior of the bee colony by stimulating memory and learning. Consumed by humans, it is a functional food with high biological and therapeutic value [42].

The samples of fresh royal jelly that we analyzed had a water content of 65.56%, protein 41.72 mg/mL, and ash 1.46%, the minimum values being observed in the case of commercial royal jelly and the maximum values in the case of that harvested by us from the hive (Table 4). In the case of protein, the differences in content were statistically significant ($p < 0.05$) between the two sources of origin. The values recorded for impurities ranged from 9.32–16.79%. The acidity of the analyzed samples ranged from 29.45 mg/100 g (from our own apiary) to 33.03 mg/100 g (freeze-dried) and the pH was in the range 3.14–3.41 (Table 4). The highest proportion of macroelements was occupied by phosphorus with 1648.38 mg, followed by potassium 1182.42 mg, magnesium 365.77 mg, calcium 200.79 mg, and sodium 182.67 mg (Table 4). We observed statistically significant differences in calcium content ($p < 0.05$) between the samples analyzed, probably due to the different geographical locations of the beekeepers and the food sources the bees had access to. The maximum values of macroelements were recorded for commercially available royal jelly, with the exception of phosphorus, for which the maximum third was found in royal jelly from the beekeeper's own apiary.

In the case of microelements, zinc (19.71 mg) and iron (17.87 mg) occupy the largest share in the composition of fresh royal jelly, followed by copper (6.26 mg), manganese (4.20 mg), and chromium (1.03 mg), the maximum values being recorded by the commercial product.

In the case of freeze-dried royal jelly, the water content was 3.33%, protein was 94.76 mg/g, and ash 2.95%. The weight of macro- and microelements was close to that recorded for fresh royal jelly (Table 4). Regarding heavy metals, nickel and lead were not detected in the analyzed samples, and cadmium was detected in the range of 0.70–1.51 ppm, with higher values observed in commercial royal jelly, both fresh and lyophilized (Table 4).

Studies by Kunugi et al. [43] showed that the major component of fresh royal jelly is water, accounting for 60–70%, while proteins represent 50% of the dry matter. Sugars have an important weight of 7.5–15%, of which 90% are represented by fructose and glucose, and lipids are present in royal jelly at a proportion of 7–18%. Sidor et al., 2021 [6] reported on fresh royal jelly from Poland with a water content of 65.4–69%, protein 10.43–18%, and pH 3.97–3.98. Balkanska and Kashamov [44] stated that Bulgarian lyophilized royal jelly has in its composition water 3.49–4.76%, dry matter 95.24–96.51%, proteins 34.09–41.80%, lipids 3.09–8.56%, sugars 24.27–32.67%, and acidity of 10.67–12.88 mg/100 g, values that confirm the results obtained by Sabatini et al. [45].

Table 4. Chemical composition of fresh and lyophilized royal jelly.

	Fresh Royal Jelly		**Lyophilized Royal Jelly**
	Own Apiary	**Market**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Water content (%)	65.56 ± 1.064 ^{a}	64.75 ± 0.079 ^{a}	3.33 ± 0.010 ^{B}
DM (%)	34.17 ± 0.987 ^{a}	35.22 ± 0.078 ^{a}	94.64 ± 2.000 ^{B}
Acidity	29.45 ± 0.045 ^{a}	31.77 ± 1.485 ^{a}	33.03 ± 1.704 ^{A}
pH	3.41 ± 0.042 ^{a}	3.14 ± 0.020 ^{b}	3.15 ± 0.050 ^{A}
Impurities (%)	9.96 ± 0.656 ^{a}	9.32 ± 0.809 ^{a}	16.79 ± 0.738 ^{B}
Protein	41.72 ± 1.005 ^{a}	36.13 ± 0.110 ^{b}	94.76 ± 5.705 ^{B}
Ash (%)	1.46 ± 0.015 ^{a}	0.93 ± 0.015 ^{b}	2.95 ± 0.050 ^{B}

Table 4. *Cont.*

	Fresh Royal Jelly		Lyophilized Royal Jelly
	Own Apiary	**Market**	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Macroelements			
Na	182.67 ± 8.457 ^{a}	187.53 ± 8.761 ^{a}	179.11 ± 4.992 ^{A}
Ca	200.79 ± 1.994 ^{a}	382.12 ± 30.716 ^{b}	1218.42 ± 1101.62 ^{A}
K	1182.42 ± 414.80 ^{a}	1659.43 ± 509.61 ^{a}	2553.17 ± 878.77 ^{A}
Mg	365.77 ± 75.038 ^{a}	423.32 ± 19.367 ^{a}	782.40 ± 109.17 ^{B}
P	1648.38 ± 343.83 ^{a}	1125.75 ± 191.62 ^{a}	2799.00 ± 890.95 ^{A}
Microelements			
Fe	17.87 ± 7.219 ^{a}	19.43 ± 7.353 ^{a}	36.34 ± 6.243 ^{A}
Mn	4.20 ± 0.636 ^{a}	5.32 ± 1.965 ^{a}	4.91 ± 0.155 ^{A}
Cu	6.26 ± 1.449 ^{a}	7.12 ± 0.820 ^{a}	15.78 ± 0.721 ^{B}
Zn	19.71 ± 5.769 ^{a}	21.95 ± 3.075 ^{a}	51.07 ± 4.617 ^{B}
Cr	1.03 ± 0.332 ^{a}	2.95 ± 4.164 ^{a}	2.68 ± 0.784 ^{A}
Contaminants			
Ni	Undetectable	undetectable	Undetectable
Cd	0.70 ± 0.169 ^{a}	1.51 ± 0.106 ^{b}	1.29 ± 0.247 ^{A}
Pb	Undetectable	undetectable	Undetectable

Results are expressed as the two-determination mean ± standard deviation (SD). Different letters indicate statistically significant differences between samples in a row ($p < 0.05$) according to the Tukey test.

The macroelement content of royal jelly studied by Sidor et al. [6] showed, as in our case, the predominant presence of phosphorus 338.4–412.1 mg, followed by potassium 321.1–357.4 mg, magnesium 44–50.4 mg, calcium 22.8–24 mg, and sodium 10.3–13.8 mg. Microelements had values of 2.07–2.58 mg in the case of zinc, 0.31–0.39 mg copper, 0.03–0.15 mg and 0.01–0.08 mg manganese, lower than those observed.

3.1.3. Chemical Composition of Propolis

Propolis is the result of harvesting and processing by bees of resinous substances from some plants, to which they add glandular secretions, wax, and residues from the digestion of pollen. In the hive, bees use propolis for sealing and sanitizing the nest and secondarily for polishing the walls of the hive and the cells, covering killed pests. Even though the chemical composition varies considerably, being correlated with the geographical area and the plants used by the bees to produce it, propolis has similar properties, including antibacterial, antifungal, antiparasitic, antiviral, anti-inflammatory, and antioxidant activities [46–48]. Raw propolis usually contains 50% vegetable resins, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other organic substances [16].

The raw propolis samples that we analyzed had a moisture content of 0.35–0.66%, the maximum value being recorded in the commercial samples, with statistically significant differences ($p < 0.05$) compared with the propolis from our own apiary (Table 5). We observed that the acidity of the commercial samples was 15.87 mg/g higher compared with the samples harvested from own apiary (5.95 mg/g) with statistically significant differences ($p < 0.05$), and a similar situation was observed for impurities (Table 5). The evaluation of macroelements revealed calcium as the main element at 840.40 mg, with significant differences between the sources of origin ($p < 0.05$), followed by magnesium 144.24 mg, phosphorus 132.00 mg, sodium 103.29 mg, and potassium 97.34 mg. The best represented microelement was iron 54.58 mg, followed by zinc 15.85 mg, manganese 4.91 mg, and copper 3.13 mg, with statistically insignificant differences between propolis

sources (Table 5). Chromium was not detected in the raw propolis samples. Among the contaminants, nickel was identified in the market propolis samples at 1.71 ppm, and cadmium at 0.24 ppm in the own hive propolis sample and 0.55 pp from the market, and lead was not detected in any sample studied.

Table 5. Chemical composition of fresh propolis and tincture.

	Fresh Propolis		Tincture of Propolis
	Market Mean ± SD	Own Apiary Mean ± SD	Mean ± SD
Water content (%)	0.66 ± 0.010 ^{a}	0.35 ± 0.010 ^{b}	96.79 ± 0.188 ^{B}
DM (%)	99.15 ± 0.175 ^{a}	98.47 ± 1.051 ^{a}	3.14 ± 0.173 ^{B}
Acidity	5.95 ± 0.010 ^{a}	3.97 ± 0.015 ^{b}	15.87 ± 0.031 ^{B}
pH	3.55 ± 0.136 ^{a}	3.92 ± 0.020 ^{b}	2.95 ± 0.025 ^{B}
Impurities (%)	12.40 ± 0.791 ^{a}	5.74 ± 0.393 ^{b}	0.09 ± 0.015 ^{B}
Ash (%)	0.94 ± 0.010 ^{a}	0.98 ± 0.025 ^{a}	0.93 ± 0.015 ^{A}
Macroelements			
Na	103.29 ± 7.636 ^{a}	110.33 ± 6.648 ^{a}	84.63 ± 4.956 ^{A}
Ca	840.40 ± 12.537 ^{a}	587.05 ± 63.01 ^{b}	437.36 ± 18.915 ^{A}
K	97.34 ± 26.467 ^{a}	128.79 ± 44.491 ^{A}	17.85 ± 3.068 ^{A}
Mg	144.24 ± 15.711 ^{a}	148.99 ± 0.084 ^{a}	58.26 ± 4.617 ^{B}
P	132.00 ± 46.669 ^{a}	152.63 ± 31.99 ^{a}	7.88 ± 3.005 ^{B}
Microelements			
Fe	54.58 ± 19.183 ^{a}	41.97 ± 0.883 ^{a}	2.03 ± 0.473 ^{B}
Mn	4.91 ± 0.650 ^{a}	5.67 ± 0.926 ^{a}	2.74 ± 0.176 ^{B}
Cu	3.13 ± 1.506 ^{a}	2.32 ± 0.120 ^{a}	2.22 ± 0.148 ^{A}
Zn	15.85 ± 6.533 ^{a}	42.73 ± 10.868 ^{a}	4.14 ± 2.418 ^{A}
Cr	Undetectable	Undetectable	0.49 ± 0.692
Contaminants			
Ni	1.71 ± 0.876 ^{a}	Undetectable	Undetectable
Cd	0.55 ± 0.212 ^{a}	0.24 ± 0.091 ^{a}	0.62 ± 0.176 ^{A}
Pb	Undetectable	Undetectable	Undetectable

Results are expressed as the two-determination mean ± standard deviation (SD). Different letters indicate statistically significant differences between samples in a row ($p < 0.05$) according to the Tukey test.

The propolis tincture studied had a moisture content of 96.79%, an acidity of 15.87 mg/g, a pH of 2.925, and an impurity percentage of 0.09% (Table 5). Calcium was the best represented macroelement at 437.36 mg, followed by sodium 84.63 mg, magnesium 58.26 mg, potassium 17.85 mg, and phosphorus 7.88 mg. Zinc was the major microelement in the propolis tincture that we analyzed, with a content of 4.14 mg, followed by manganese 2.74 mg, copper 2.22 mg, and iron 2.03 mg. Of the heavy metals analyzed, cadmium was identified at a concentration of 0.62 ppm, and nickel and lead were below the detection limit.

The studies carried out so far have shown that there are correlations between the chemical composition of propolis and the plants used by bees for its production [17]. The type of soil and its parameters, the botanical origin of the samples, the geographical area, and the climatic conditions can determine differences in the mineral profile of propolis from different areas [49]. The mineral contents of the propolis samples evaluated have values close to those reported by Cvek et al. [50] in the case of propolis from Croatia, and Tosic et al. [49] for propolis from Serbia, the order of macrominerals being Ca > K > Mg > P

> Na, while in the case of microelements, the largest share is occupied by iron, followed by zinc. The authors reported the following values for the 25 propolis samples analyzed: calcium 627–1168 mg/kg, potassium 324–1157 mg/kg, magnesium 157 mg/kg, phosphorus 134–422 mg/kg, sodium 63.5–256 mg/kg, 116–284 mg/kg, zinc 19.2–241 mg/kg, manganese 6.1–14.36 mg/kg, and copper 2.22–8.70 mg/kg [49].

3.2. Antioxidant Activity by DPPH Method

To evaluate the radical scavenging activity using the DPPH method, five concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1 mg/mL and 0.5 mg/mL) were prepared from each ethanolic extract of the nine samples tested (Table 6). In parallel, the antioxidant activity of five ascorbic acid solutions prepared at different concentrations (0.06–0.16 mg/mL) was also evaluated as a positive control, resulting in 94.54% inhibition at the highest concentration tested (0.16 mg/mL). The IC50 (concentration of each extract causing 50% DPPH inhibition) was then calculated and expressed in mg/mL (Table 6, Figure 1).

Table 6. The DPPH radical scavenging activity (% inhibition) of ethanolic extracts vs. ascorbic acid.

Concentration (mg/mL)	Samples									Ascorbic Acid	
	PS	PC	PT	LMS	LML	LMC	AS	ALC	AP	Concentration (mg/mL)	% Inhibition
	% Inhibition										
10	63.65	58.91	82.93	84.57	84.09	80.77	96.00	97.00	80.08	0.16	94.54
5	52.07	47.89	80.52	64.45	80.02	71.33	87.51	86.40	77.68	0.14	82.32
2.5	46.23	44.50	64.85	54.44	75.06	65.76	84.36	84.70	70.38	0.10	65.24
1	30.34	33.55	56.86	50.25	53.76	55.31	55.77	50.90	50.89	0.08	45.48
0.5	24.89	21.76	37.77	36.34	36.39	37.15	32.30	30.09	30.40	0.06	25.22

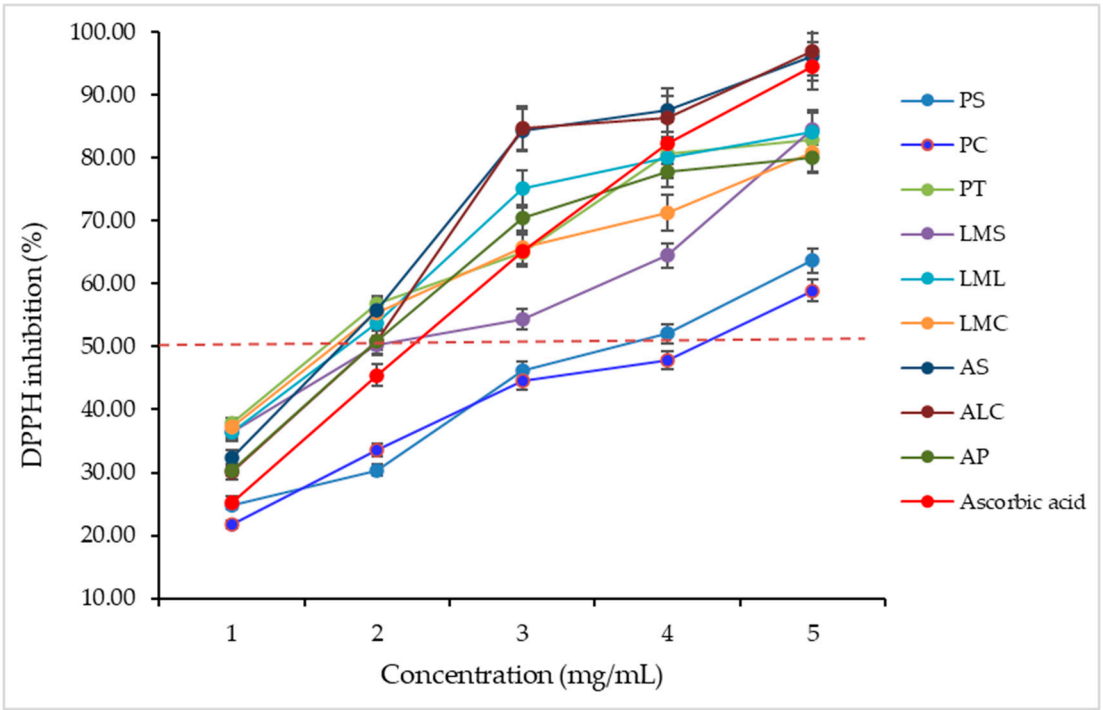


Figure 1. Concentration dependency of radical scavenging activity of the samples.

The maximum radical scavenging activity for all samples was recorded at the highest concentration (10 mg/mL), as shown in Table 6. In the case of AS and ALC samples, higher

values were obtained than in the case of the control sample, ascorbic acid, analyzed in the concentration range 0.06–0.16 mg/mL.

At the 5 mg/mL concentration, except for the PS, PC, and LMS samples, the other samples gave values similar to those recorded for ascorbic acid.

The next two lower concentrations (2.5 mg/mL and 1 mg/mL) still showed a high percentage of DPPH inhibition. This was true for all samples except PS and PC. However, the values recorded are comparable to those recorded for ascorbic acid. At the lowest concentration tested (0.3 mg/mL), the antioxidant activity showed a significant decrease for all the samples, with values comparable to those recorded for ascorbic acid and even higher for some of the samples.

The DPPH inhibition percentage at 10 mg/mL concentration was >90% for AS and ALC, >80% for PT, LMS, LML, CML, and AP, and >50% for PS and PC. At 5 mg/mL, it was >80% for PT, CML, AS, and ALC, >70% for CML and AP, >50% for PS and LMS, and <50% for PC. At a concentration of 2.5 mg/mL, >80% was recorded for AS and ALC, >50% for PT, LMS, LML, and AP, and PS and PC had DPPH values < 50%. At 1 mg/mL, values > 50% were recorded for all samples except PS and PC. At the lowest concentration, values > 30% were recorded except for the PS and PC samples.

Table 6 shows the IC50 values (the extract concentration that determines 50% DPPH inhibition) obtained for the samples analyzed, in comparison with the value obtained for the control sample, ascorbic acid.

IC50 values (Table 7) ranged from 1669 mg/mL for the AS sample (highest antioxidant capacity) to 3979 mg/mL (lowest antioxidant capacity) in the case of the PC sample. The variation of the IC50 values of the analyzed samples is shown in Figure 1.

Table 7. The IC50 values of samples vs ascorbic acid.

Samples	PS	PC	PT	LMS	LML	LMC	AS	ALC	AP	Ascorbic Acid
IC50 ± SEM (mg/mL)	3.661 ± 0.002	3.979 ± 0.002	1.720 ± 0.001	2.276 ± 0.002	1.696 ± 0.001	1.832 ± 0.001	1.669 ± 0.001	1.830 ± 0.001	2.058 ± 0.001	2.284 ± 0.001
R^2	0.9788	0.9752	0.9488	0.9497	0.9067	0.9556	0.9027	0.904	0.9017	0.9913
Hill Slope	9.925	8.864	11.398	11.066	12.166	10.326	15.914	16.932	12.615	17.548

Results are expressed as the three-determination mean ± standard deviation (SD).

Figure 1 shows the dependence of free radical reducing activity on the concentration of the extracts analyzed. A high antioxidant potential is shown by samples that register high inhibition at low concentration.

From Figure 1 it can be seen that for all the samples analyzed, the inhibition recorded is directly proportional to the concentration. The ascending order of the minimum concentrations determining 50% DPPH inhibition compared with ascorbic acid (control) was as follows: PT < LMC < AS < LML < ALC < AP < LMS < ascorbic acid < LML < PC.

IC50 values ranging from 0.3 mg/mL to 5.6 mg/mL were reported by Mărghitaş et al. [51] for 13 ethanolic extracts of propolis collected from beehives in Transylvania. Chinese propolis was analyzed by Sun et al. [52] and its antioxidant activity was reported with IC50 values ranging from 0.633 mg/mL to 13.798 mg/mL for different propolis extracts. Guzman-Gutierrez et al. [53] reported strong DPPH scavenging activity (IC50 = 16.55 ± 0.87 µg/mL) in a study carried out on extracts of Mexican propolis and ethyl acetate. Strong antioxidant activity was reported by Belfar et al. [54] for four Algerian propolis methanolic extracts with IC50 values between 0.007 and 0.066 mg/mL, but lower than control ascorbic acid (0.184 mg/mL).

Brazilian propolis has been reported to have antioxidant activity, with DPPH scavenging activity varying from 23.7% to 43.5% at different doses, using ascorbic acid as

a positive control [55], with values similar to ours at concentrations of 0.5 (mg/mL). A study by Kumazawa et al. [56] on propolis extracts from 14 countries around the world evaluated their antioxidant activity and reported a wide range of DPPH radical scavenging activity (from 10% to 90%), values similar to those obtained for the three propolis samples. According to that study, the strongest results were obtained by propolis from countries such as Australia, China, Hungary, and New Zealand. In the case of propolis originating in India, IC50 determinations for 10 ethanolic extracts varied between 0.3334 mg/mL and 0.6008 mg/mL, while for ascorbic acid the value was 0.2849 mg/mL [57]. IC50 values ranging from 0.043 to 0.269 mg/mL for Korean propolis were reported by Wang et al. [58] for 20 samples investigated.

4. Conclusions

The chemical composition of the analyzed bee products, apilarnil, royal jelly, and propolis, shows great variability, being conditioned by the form of presentation and the source of origin. In general, commercial bee products had the best results in terms of chemical composition, including higher content of macro and microelements. All analyzed samples had values of acidity, pH, and content of impurities within the limits allowed by the quality standards.

Apilarnil originating from our own beehive recorded the best results for antioxidant activity, being followed by propolis tincture, and fresh propolis originating from our own beehive, in comparison with similar beekeeping products taken from the market. The commercial royal jelly showed better antioxidant activity than that from our apiary.

Author Contributions: All authors contributed to the study conception and design. Conceptualization original draft preparation, D.M., S.P., I.C., D.O., E.A. and I.R.; methodology, formal analysis, D.M., S.P., I.C., D.O., E.A., I.R., A.B., A.G., M.D. and T.V.; statistical analysis, E.S.; review and editing and validation, D.M., S.P., I.C., D.O. and E.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research paper is supported by the project Institutional Development Fund (FDI), domain 6, by the National Council for the Financing of Higher Education (CNFIS), CNFIS-FDI-2023-F-0307.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The report of the analyses performed on the samples in this paper can be found at the Interdisciplinary Research Platform (PCI) belonging to the University of Life Sciences "King Mihai I" Timisoara. https://www.usab-tm.ro/ro/centre-si-laboratoare-de-cercetare-10067/centre-de-cercetare-11715, further inquiries can be directed to the authors (ileanacocan@usvt.ro or ersiliaalexa@usvt.ro).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kolayli, S.; Keskin, M. Natural bee products and their apitherapeutic applications. *Stud. Nat. Prod. Chem.* **2020**, *66*, 175–196. [CrossRef]
2. Bartkiene, E.; Lele, V.; Sakiene, V.; Zavistanaviciute, P.; Zokaityte, E.; Dauksiene, A.; Jagminas, P.; Klupsaite, D.; Bliznikas, S.; Ruzauskas, M. Variations of the antimicrobial, antioxidant, sensory attributes and biogenic amines content in Lithuania-derived bee products. *LWT Food Sci. Technol.* **2020**, *118*, 108793. [CrossRef]
3. Viuda-Martos, M.; Ruiz-Navajas, Y.; Fernández-López, J.; Pérez-Alvarez, J.A. Functional properties of honey, propolis, and royaljelly. *J. Food Sci.* **2008**, *73*, R117. [CrossRef] [PubMed]
4. Martinello, M.; Mutinelli, F. Antioxidant Activity in Bee Products: A Review. *Antioxidants* **2021**, *10*, 71. [CrossRef]
5. Iliesiu, N.V. *Apilarnil*; Editura Apimondia: Bucuresti, Romania, 1991; p. 33.
6. Sidor, E.; Miłek, M.; Zaguła, G.; Bocian, A.; Dżugan, M. Searching for Differences in Chemical Composition and Biological Activity of Crude Drone Brood and Royal Jelly Useful for Their Authentication. *Foods* **2021**, *10*, 2233. [CrossRef] [PubMed]
7. Bogdanov, S. Royal Jelly, Bee Brood: Composition, Health, Medicine: A Review. Bee Product Science. 2015. Available online: https://www.bee-hexagon.net/ (accessed on 14 February 2023).

8. Lima, W.G.; Brito, J.C.; Da Cruz Nizer, W.S. Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). *Phytother. Res.* **2021**, *35*, 743–750. [CrossRef]
9. Bărnuţiu, L.I.; Mărghitaş, L.A.; Dezmirean, D.S.; Mihai, C.M.; Bobiş, O. Chemical composition and antimicrobial activity of Royal Jelly-REVIEW. *Sci. Pap. Anim. Sci. Biotechnol.* **2011**, *44*, 67–72.
10. Ahmad, S.; Campos, M.G.; Fratini, F.; Altaye, S.Z.; Li, J. New Insights into the Biological and Pharmaceutical Properties of Royal Jelly. *Int. J. Mol. Sci.* **2020**, *21*, 382. [CrossRef]
11. Bengü, A.Ş.; Adnan, A.Y.N.A.; Özbolat, S.; Abdullah, T.U.N.Ç.; Aykutoğlu, G.; Çiftci, M.; Darendelioğlu, E. Content and antimicrobial activities of bingol royal jelly. *Türk Tarım Doğa Bilim. Derg.* **2020**, *7*, 480–486. [CrossRef]
12. Yükünç, G.O. Royal jelly: Proteins and peptides. *J. Apitherapy Nat.* **2020**, *2*, 59–70. [CrossRef]
13. Shen, L.; Liu, D.; Li, M.; Jin, F.; Din, M.; Parnell, L.D.; Lai, C.Q. Mechanism of action of recombinant acc-royalisin from royal jelly of Asian honeybee against gram-positive bacteria. *PLoS ONE* **2012**, *7*, e47194. [CrossRef] [PubMed]
14. Pătruică, S. *Biotehnologii în Apicultură și Sericicultură*; Editura Eurobit: Timișoara, Romania, 2021; pp. 39–62.
15. Przybyłek, I.; Karpiński, T.M. Antibacterial Properties of Propolis. *Molecules* **2019**, *24*, 2047. [CrossRef] [PubMed]
16. Huang, S.; Zhang, C.-P.; Wang, K.; Li, G.Q.; Hu, F.-L. Recent Advances in the Chemical Composition of Propolis. *Molecules* **2014**, *19*, 19610–19632. [CrossRef] [PubMed]
17. Wieczorek, P.P.; Hudz, N.; Yezerska, O.; Horčinová-Sedláčková, V.; Shanaida, M.; Korytniuk, O.; Jasicka-Misiak, I. Chemical Variability and Pharmacological Potential of Propolis as a Source for the Development of New Pharmaceutical Products. *Molecules* **2022**, *27*, 1600. [CrossRef] [PubMed]
18. Pavlovic, R.; Borgonovo, G.; Leoni, V.; Giupponi, L.; Ceciliani, G.; Sala, S.; Bassoli, A.; Giorgi, A. Effectiveness of Different Analytical Methods for the Characterization of Propolis: A Case of Study in Northern Italy. *Molecules* **2020**, *25*, 504. [CrossRef] [PubMed]
19. Huang, S.; Zhang, C.-P.; Li, G.Q.; Sun, Y.-Y.; Wang, K.; Hu, F.-L. Identification of Catechol as a New Marker for Detecting Propolis Adulteration. *Molecules* **2014**, *19*, 10208–10217. [CrossRef] [PubMed]
20. Martinotti, S.; Pellavio, G.; Laforenza, U.; Ranzato, E. Propolis Induces AQP3 Expression: A Possible Way of Action in Wound Healing. *Molecules* **2019**, *24*, 1544. [CrossRef] [PubMed]
21. Tahira, F.; Akhlaq, A.F. Beneficial effects of propolis on human health and neurological diseases. *Front. Biosci.* **2012**, *4*, 779–793.
22. Ali, A.M.; Kunugi, H. Propolis, Bee Honey, and Their Components Protect against Coronavirus Disease 2019 (COVID-19): A Review of In Silico, In Vitro, and Clinical Studies. *Molecules* **2021**, *26*, 1232. [CrossRef]
23. ALaerjani, W.M.A.; Khan, K.A.; Al-Shehri, B.M.; Ghramh, H.A.; Hussain, A.; Mohammed, M.E.A.; Imran, M.; Ahmad, I.; Ahmad, S.; Al-Awadi, A.S. Chemical Profiling, Antioxidant, and Antimicrobial Activity of Saudi Propolis Collected by Arabian Honey Bee (Apis mellifera jemenitica) Colonies. *Antioxidants* **2022**, *11*, 1413. [CrossRef]
24. Xu, X.; Pu, R.; Li, Y.; Wu, Z.; Li, C.; Miao, X.; Yang, W. Chemical Compositions of Propolis from China and the United States and their Antimicrobial Activities Against Penicillium notatum. *Molecules* **2019**, *24*, 3576. [CrossRef]
25. An, S.-H.; Ban, E.; Chung, I.-Y.; Cho, Y.-H.; Kim, A. Antimicrobial Activities of Propolis in Poloxamer Based Topical Gels. *Pharmaceutics* **2021**, *13*, 2021. [CrossRef]
26. Botteon, C.E.A.; Silva, L.B.; Ccana-Ccapatinta, G.V.; Silva, T.S.; Ambrosio, S.R.; Veneziani, R.C.S.; Bastos, J.K.; Marcato, P.D. Biosynthesis and characterization of gold nanoparticles using Brazilian red propolis and evaluation of its antimicrobial and anticancer activities. *Sci. Rep.* **2021**, *11*, 1974. [CrossRef] [PubMed]
27. Ulag, S.; Ilhan, E.; Demirhan, R.; Sahin, A.; Yilmaz, B.K.; Aksu, B.; Sengor, M.; Ficai, D.; Titu, A.M.; Ficai, A.; et al. Propolis-Based Nanofiber Patches to Repair Corneal Microbial Keratitis. *Molecules* **2021**, *26*, 2577. [CrossRef] [PubMed]
28. Salatino, A. Perspectives for Uses of Propolis in Therapy against Infectious Diseases. *Molecules* **2022**, *27*, 4594. [CrossRef] [PubMed]
29. Yosri, N.; Abd El-Wahed, A.A.; Ghonaim, R.; Khattab, O.M.; Sabry, A.; Ibrahim, M.A.A.; Moustafa, M.F.; Guo, Z.; Zou, X.; Algethami, A.F.M.; et al. Anti-Viral and Immunomodulatory Properties of Propolis: Chemical Diversity, Pharmacological Properties, Preclinical and Clinical Applications, and In Silico Potential against SARS-CoV-2. *Foods* **2021**, *10*, 1776. [CrossRef]
30. Chavda, V.P.; Chaudhari, A.Z.; Teli, D.; Balar, P.; Vora, L. Propolis and Their Active Constituents for Chronic Diseases. *Biomedicines* **2023**, *11*, 259. [CrossRef] [PubMed]
31. *SR 784-3:2009*; Honey. Part 3: Analysis Methods. ASRO—Asociatia de Standardizare din România: Bucharest, Romania, 2009.
32. Wagh, V.D. Propolis: A wonder bees product and its pharmacological potentials. *Adv. Pharmacol. Pharm. Sci.* **2013**, *2023*, 308249. [CrossRef] [PubMed]
33. Gergen, I. *Agri-Food Products Analysis*; EUROSTAMPA Publishing House: Timisoara, Romania, 2004; 316p, ISBN 973-687-271-8.
34. Pătruică, S.; Alexa, E.; Obiștioiu, D.; Cocan, I.; Radulov, I.; Berbecea, A.; Lazăr, R.N.; Simiz, E.; Vicar, N.M.; Hulea, A.; et al. Chemical Composition, Antioxidant and Antimicrobial Activity of Some Types of Honey from Banat Region, Romania. *Molecules* **2022**, *27*, 4179. [CrossRef]
35. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein Measurement with Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 256–275. [CrossRef]
36. Preda, G.; Dragomirescu, M.; Peter, F. *Biocatalizatori Enzimatici. Obţinere, Caracterizare, Aplicaţii*; Editura Mirton: Timişoara, Romania, 2003; pp. 29–31.

37. Cadariu, A.I.; Cocan, I.; Negrea, M.; Alexa, E.; Obistioiu, D.; Hotea, I.; Radulov, I.; Poiana, M. Exploring the potential of tomato processing by-product as a natural antioxidant in nitrite-free reformulated sausages. *Sustainability* **2022**, *14*, 11802. [CrossRef]
38. Margaoan, R.; Marghitas, L.A.; Dezmirean, D.S.; Bobis, O.; Bonta, V.; Catana, C.; Urcan, A.; Mureşan, C.I.; Margin, M.G. Comparative study on quality parameters of royal jelly, apilarnil and queen bee larvae triturate. *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Anim. Sci. Biotechnol.* **2017**, *74*, 51–58. [CrossRef] [PubMed]
39. Kim, S.G.; Woo, S.O.; Bang, K.W.; Jang, H.R.; Han, S.M. Chemical composition of drone pupa of Api mellifera and its nutritional evaluation. *Korean J. Apic.* **2018**, *33*, 17–23.
40. Kim, J.E.; Kim, D.I.; Koo, H.Y.; Kim, H.J.; Kim, S.Y.; Lee, Y.B.; Kim, J.S.; Kim, H.H.; Moon, J.H.; Choi, Y.S. Analysis of nutritional compounds and antioxidant effect of freeze-dried powder of the honey bee (*Apis mellifera* L.) drone (pupal stage). *Korean J. Appl. Entomol.* **2020**, *59*, 265–275.
41. Choi, Y.S.; Lee, M.L.; Lee, M.Y.; Kim, H.K.; Lee, K.G.; Yeo, J.H.; Woo, S.O. Management for high quality drone products. *Korean J. Apic.* **2009**, *24*, 1–7.
42. Botezan, S.; Baci, G.-M.; Bagameri, L.; Pașca, C.; Dezmirean, D.S. Current Status of the Bioactive Properties of Royal Jelly: A Comprehensive Review with a Focus on Its Anticancer, Anti-Inflammatory, and Antioxidant Effects. *Molecules* **2023**, *28*, 1510. [CrossRef]
43. Kunugi, H.; Mohammed Ali, A. Royal Jelly and Its Components Promote Healthy Aging and Longevity: From Animal Models to Humans. *Int. J. Mol. Sci.* **2019**, *20*, 4662. [CrossRef]
44. Balkanska, R.; Kashamov, B. Composition and physico-chemical properties of lyophilized royal jelly. *Uludag Bee J.* **2011**, *11*, 114–117.
45. Sabatini, A.G.; Marcazzan, G.L.; Caboni, M.F.; Bogdanov, S.; Almeida-Muradian, L.B. Quality and standardisation of royal jelly. *J. ApiProd. ApiMed. Sci.* **2009**, *1*, 1–6. [CrossRef]
46. Schnitzler, P.; Neuner, A.; Nolkemper, S.; Zundel, C.; Nowack, H.; Sensch, K.H.; Reichling, J. Antiviral activity and mode of action of propolis extracts and selected compounds. *Phytother. Res.* **2010**, *24* (Suppl. S1), S20–S28. [CrossRef]
47. Ramanauskiene, K.; Inkeniene, A. Propolis oil extract: Quality analysis and evaluation of its antimicrobial activity. *Nat. Prod. Res.* **2011**, *25*, 1463–1468. [CrossRef] [PubMed]
48. De Freitas, M.C.D.; de Miranda, M.B.; de Oliveira, D.T.; Vieira-Filho, S.A.; Caligiorne, R.B.; de Figueiredo, S.M. Biological activities of red propolis: A review. Recent Pat. *Endocr. Metab. Immun. Drug Discov.* **2017**, *11*, 3–12. [CrossRef] [PubMed]
49. Tosic, S.; Stojanovic, G.; Mitic, S.; Pavlovic, A.; Alagic, S. Mineral composition of selected Serbian propolis samples. *J. Apic. Sci.* **2017**, *61*, 5–15. [CrossRef]
50. Cvek, J.; Medić-Šarić, M.; Vitali, D.; Vedrina-Dragojević, I.; Šmit, Z.; Tomić, S. The content of essential and toxic elements in Croatian propolis samples and their tinctures. *J. Apic. Res.* **2008**, *47*, 35–45. [CrossRef]
51. Mărghitaş, L.A.; Dezmirean, D.; Moise, A.; Mihai, C.M.; Laslo, S.L. DPPH Method for Evaluation of Propolis Antioxidant Activity. *Bull. UASVM Anim. Sci. Biotechnol.* **2009**, *66*, 253–258.
52. Sun, C.; Wu, Z.; Wang, Z.; Zhang, H. Effect of Ethanol/Water Solvents on Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts. *Evid. Based Complement. Altern. Med.* **2015**, *2015*, 595393. [CrossRef]
53. Guzman-Gutierrez, S.L.; Nieto-Camacho, A.; Castillo-Arellano, J.I.; Huerta-Salazar, E.; Hernandez-Pasteur, G.; Silva-Miranda, M.; Arguello-Najera, O.; Sepulveda-Robles, O.; Espitia, C.I.; Reyes-Chilpa, R. Mexican Propolis: A Source of Antioxidants and Anti-Inflammatory Compounds, and Isolation of a Novel Chalcone and epsilon-Caprolactone Derivative. *Molecules* **2018**, *23*, 334. [CrossRef] [PubMed]
54. Belfar, M.L.; Lanez, T.; Rebiai, A.; Ghiaba, Z. Evaluation of Antioxidant Capacity of Propolis Collected in Various Area of Algeria Using Electrochemical Techniques. *Int. J. Electrochem. Sci.* **2015**, *10*, 9641–9651. [CrossRef]
55. Nagai, T.; Inoue, R.; Inoue, H.; Suzuki, N. Preparation and antioxidant properties of water extract of propolis. *Food Chem.* **2003**, *80*, 29–33. [CrossRef]
56. Kumazawa, S.; Hamasaka, T.; Nakayama, T. Antioxidant activity of propolis of various geographic origins. *Food Chem.* **2004**, *84*, 325–498. [CrossRef]
57. Ramnath, S.; Venkataramegowda, S. Antioxidant Activity of Indian Propolis—An In Vitro Evaluation. *Int. J. Pharmacol. Phytochem. Ethnomed.* **2016**, *5*, 79–85. [CrossRef]
58. Wang, X.; Sankarapandian, K.; Cheng, Y.; Ok Woo, S.; Kwon, H.W.; Perumalsamy, H.; Ahn, Y.J. Relationship between total phenolic contents and biological properties of propolis from 20 different regions in South Korea. BMC Complement. *Altern. Med.* **2016**, *16*, 12.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.