European Food Research and Technology (2023) 249:2175–2187
https://doi.org/10.1007/s00217-023-04284-z

ORIGINAL PAPER

Palynological, chemical, antimicrobial, and enzyme inhibition properties of *Cannabis sativa* L. propolis

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Received: 23 February 2023 / Revised: 25 April 2023 / Accepted: 29 April 2023 / Published online: 16 May 2023

Abstract

In this study, both chemical characterization and biological activities of propolis samples obtained from *Cannabis sativa* L. (cannabis, hemp, marijuana) isolated colonies (research group) and obtained from colonies at a distance where the bee cannot fly to the isolation area (control group) were determined. Palynological analyses of propolis samples obtained from the research and control group colonies were made and the botanical origin of the cannabis plant and other plants in the research area and the plants in the control group were determined. Bioactive component analysis of propolis extracts was determined by the GC–MS technique. The inhibition effect of propolis samples on some enzymes of medical importance and their antimicrobial effects against many pathogenic microorganisms were also investigated. Palynological analyses showed that honey bees prefer cannabis plants (11.11%) when collecting propolis, and the dry matter percentage (94.6 ± 1.8%), balsam (46.2 ± 5.2%), and wax content (4.8 ± 0.5%), total phenolic (26.44 ± 1.08 mg GAE/mL) and total flavonoid (2.03 ± 0.11 mg QE/mL) content and antioxidant capacity (88.16 ± 2.27 µM $FeSO_4 \cdot 7H_2O/mL^{-1}$) of the research group propolis samples containing hemp were found to be higher than the control group. Also, it was determined that the research group propolis sample contains cannabinoid derivatives (cannabidivarinic acid, 0.29%; delta (1)-tetrahydrocannabinolic acid, 0.59%; cannabidiolic acid, 0.48%), which are active compounds of the cannabis plant. Also, a high inhibition effect was detected on some enzymes of medical importance in propolis samples obtained from cannabis group colonies. In addition, the research group propolis samples also showed a high antimicrobial effect against many pathogenic microorganisms.

Keywords Antimicrobial activity · *Apis mellifera* L. · *Cannabis sativa* L. · Enzyme inhibition · Palynology · Propolis

Introduction

Cannabis sativa L. (cannabis, hemp, marijuana) is an annual plant belonging to the Cannabaceae family. It is the oldest source of herbal raw materials in human history. Its homeland is Central Asia. It grows and cultivates in temperate and tropical regions and is a versatile plant. Its leaves are used in medicine and cosmetics [1]. The seeds of hemp are used as foodstuffs because of their high oil content and are highly nutritious. Its seeds are also used in soap-making and paint production [1].

Industrial hemp is used for a variety of commercial products, including paper, textiles, clothing, biodegradable plastics, biofuel, food, and animal feed. Wind-pollinated, dioecious, and hardy cannabis plants produce large amounts of pollen attractive to bees [2]. Hemp flowers release an abundance of pollen during a period of native and agricultural floral dearth late in the summer [3, 4] therefore, hemp pollen

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may offer a vital subsistence resource to bees at a point in the season when they are resource-limited [3]. The most suitable harvest time for cannabis to be used in the fiber industry ends with the release of pollen by the males. Harvesting for seed is done 4–6 weeks after fiber harvest when 60% of the seeds are ripe. Fiber hemp is harvested within 70–90 days after planting [5].

The composition of cannabis is cannabinol, cannabidiol, cannabigerol, cannabidiolic acid, cannabichromene, and resin. It is used in the treatment of many diseases such as cancer, stomach, antispasmodic, narcotic, analgesic, stimulant, aphrodisiac, sedative, viral, and metabolic diseases. High doses cause mental euphoria, hallucinations, and memory loss [6]. It has been used in traditional Chinese medicine to treat pain, tetany and convulsions, gout, mania, insomnia, breathing and cough, headache, menstrual irregularities, itching, and anemia. In traditional Indian medicine, its stimulant, digestive and analgesic, sedative, spasmolytic, diuretic, aphrodisiac, glaucoma treatment, skincare, antiviral, and antiparasitic effects were used [7]. Cannabidiol, an essential oil component derived from hemp, has shown effective results in controlling *Nosema ceranae* infection in honeybees [8].

Hemp is an important source of resin [9]. Honey bees carry the resin they collect from plants to the hive and turn it into propolis, a resinous substance. Propolis is the only natural mixture that honey bees use to defend their hives physically and chemically. More than 300 components have been identified in propolis [10, 11]. The chemical composition of propolis varies depending on the plant, region, season, and colony [12, 13]. With many studies, the chemical structure of some propolis types such as European poplar propolis, Brazilian green and red propolis has been well clarified and standardized [11]. Knowing the plant sources where propolis can be collected is important in terms of establishing chemical standardization besides its scientific aspect [12].

Propolis is a pharmaceutical bioactive natural bee product that contains antioxidant, antimicrobial, antitumoral, and anti-inflammatory properties. Scientific studies have reported that in addition to volatile components such as terpene, terpenoid, and sesquiterpene in the composition of propolis, numerous polyphenolic compounds it contains are responsible for biological activity [14, 15]. It is used as a natural remedy for embalming, increasing the body's defense mechanism against infections and closing wounds, since its anti-decay feature has been known since ancient times [16, 17]. Cardiovascular and blood circulation system (anemia), respiratory tract infections, treatment of dental diseases, treatment of skin diseases (tissue regeneration, ulcer, eczema), wound healing, mycosis, mucous membrane infections and lesions, cancer treatment, immune system treatment. The use of propolis as a drug in the fields of health and digestive disorders is quite common [18–20].

The antioxidant and antimicrobial effects of propolis make it attractive to use in the food industry. One of the most important properties of propolis that contributes to the durability of food products is its antimicrobial activity. Many scientific studies have been conducted on the effect of propolis on various bacteria, fungi, viruses, and other microorganisms [21–25]. This important feature of propolis has paved the way for its use as a food additive.

The cannabis plant is pharmacologically important. It is a medicinal plant that is widely used as a psychoactive drug in traditional and complementary medicine [26]. Studies have shown that the plant exhibits antioxidant properties through the components it contains [27]. There is no data in the literature on the chemical composition of propolis obtained from the plant [26], which predominantly contains components such as cannabidiol, cannabidiolic acid, cannabigerol, and cannabigerolic acid.

In this study, *C. sativa* L. based propolis samples were obtained by controlled conditions. The botanical origin of the obtained propolis samples from the research and control group colonies were determined by making palynological analyses. Then, the chemical and biochemical characterization of propolis samples and the antimicrobial activities of these propolis samples were determined and *C. sativa* L. propolis properties were identified.

Materials and methods

Obtaining cannabis plants

This research was carried out in Malatya city in Turkey in the spring of 2021. *C. sativa* L. was planted in the field for scientific studies on a five-decare application area of Malatya Turgut Özal University Faculty of Agriculture as plant material. Flowering in the cannabis plant started in the first week of August and ended in the first week of October. Malatya Turgut Özal University Campus, where cannabis is cultivated, is within the borders of Malatya province Battalgazi. The campus is spread over a wide area, and it is an area rich in flora, where many plants in culture and wild form are found and grown. There are plants such as oak, yellow pine, poplar, willow, thyme, euphorbia, oleaster, blackberry, and wild rose in this area. In addition, in agricultural areas, there are vegetables (tomato, pepper, cabbage, etc.) and orchards (apricot, cherry, walnut, apple, etc.), vineyards, and nurseries as important agricultural products.

Depending on the climate, steppe vegetation is seen in the Hekimhan locality, where control group colonies that do not cultivate cannabis are accommodated. There are oak groves in these places. Wild fruit trees and poplars and willows in springs and valleys are added to this cover.

Honey bee colonies

The honey bee material of the study was formed by colonies belonging to Malatya Turgut Özal University Bee and Bee Products Development, Application, and Research Center. In the study, 40 honey bee colonies in Langstroth-type hives were used. 20 honey bee colonies were located in Hekimhan (latitude: 38.817, longitude: 37.933, 38° 49′ 1″ North, 37° 55′ 59″ East) as a control group, and 20 honey bee colonies were located in Battalgazi (Latitude: 38.4234, Longitude: 38.3656, 38° 25′ 24″ North, 38° 21′ 56″ East) as a research group. The map of the locations where the research was carried out is given in Fig. 1. Colonies are equidistant in brood and food. In this synchronization, it was ensured that each colony had 6 frames of bees and 4 frames of brood. The queens of the colonies were replaced with one-year-old Caucasian honey bee (*Apis mellifera caucasica*) queens reared from the same colony.

Palynological analyses

Palynological analyzes were performed at Hacettepe University Bee and Bee Products Application and Research Center (HARÜM).

Bayram et al. [28] methodology was followed and 1 g of propolis sample was weighed and mixed with ethanol-ether-acetone (1:1:1) according to this method. This mixture was vortexed and filtered through a 0.3 mm perforated strainer into another centrifuge tube. This suspension was then centrifuged at 3500 rpm for 20 min. The supernatant was then poured out and only the sediment side was used for palynological analysis. For this purpose, 1–2 mm^3 glycerin gelatin matrix with basic fuchsin was taken with a sterile needle, and a residue piece with glycerin gelatin matrix was turned into slides. The slide in this form was heated at 30–40 °C to dissolve the glycerin gelatin matrix; then it is covered with an 18 × 18 mm^2 lamella. Propolis slides were examined with a light microscope (Nikon Eclipse E400, Japan) for pollen analysis 20 ×, 40 ×, and 100 × objectives were used to identify pollen grains.

In the diagnosis of pollen, various sources, the collection of pollen preparations of the Hacettepe University Department of Biology, and reference pollen preparations prepared from plants collected from the research area were used. Plant identifications were made by counting 200 pollen in the preparations. Plant pollens, whose frequency was determined by diagnosis, were classified as dominant (D) (> 45%), secondary (S) (16–45%), minor (M) (3–15%), and trace (T) (< 3%).

Propolis characterization

Extraction of propolis

Raw propolis samples were collected from propolis traps in the form of plastic grid cover boards from the hives placed in the cannabis-grown research group and non-hemp-grown control group areas. The traps in which the propolis samples were collected were kept in the hives until the cannabis harvest. After the cannabis plant was harvested, propolis traps were collected from the research and control colonies, and propolis was harvested from the colonies.

Raw propolis samples obtained from research and control group colonies were frozen, ground, and blended, and each sample was mixed homogeneously within itself. Thus, propolis samples to be analyzed were obtained. 90 g of the

Fig. 1 Malatya city of Turkey. The research area is in Battalgazi and the control group is in Hekimhan

obtained samples were individually weighed and extracted with 900 mL of 70% ethyl alcohol using the maceration technique (200 rpm constant speed and stirring for 24 h at room temperature). At the end of the relevant period, the extract was filtered through Whatman no 1 filter paper [29]. The extracts were stored at room temperature and in the dark throughout the research.

Dry matter amount

% dry matter amount was made according to the method stated by Woisky and Slantino [13]. For this purpose, a 10 g raw propolis sample was weighed with weighing cups that came to a constant weight. It was dried at 105 °C for 5 h. At the end of the relevant time, the samples were taken into a desiccator and weighed after reaching room temperature. % dry matter content was calculated by using the difference between the first weighing and the final weighing [29]. Analyzes were made in 3 replications and the results were given as the mean value.

Balsam quantity

The amount of balsam was determined according to the ethyl alcohol extraction method [25]. In order to determine the ratio of balsam, 2 mL of propolis extract was evaporated and the amount of resulted solid was quantified until reaching a constant weight. The amount of balsam of the extract was calculated and expressed as a percentage value. Analyzes were made in 3 replications and the results were given as the mean value.

The wax amount

The wax value of raw propolis was determined by making some changes in the method specified in Feas et al. [30]. For this purpose, ground raw propolis samples were used. 3 g of propolis samples were weighed separately and 15 mL of absolute methanol was added to them. The resulting mixture was shaken for 2 h in a shaker at a constant speed and room temperature. Then the mixture was kept in the deep freezer (− 18 °C) overnight. The wax adsorbed at the top of the solution was carefully collected from the solution and filtered. The % wax value was calculated over the difference between the last weighing and the first weighing of filter paper [29] Analyzes were made in 3 replications and the results were given as the mean value.

Determination of the total amount of phenolic contents (TPC)

The total phenolic content of propolis extracts was determined using the Folin method [31, 32]. A calibration curve was prepared using the gallic acid standard and results were expressed as mg gallic acid equivalent (GAE)/mL propolis extract. Analyzes were made in 3 replications and the results were given as the mean value.

Total flavonoid contents (TFC)

The flavonoid amount was determined according to Fukumoto and Mazza [33]. Quercetin was used at different concentrations (0.25; 0.125; 0.0625; 0.03125; 0.015625 and 0.0078125 mg/mL) as a standard, and the total flavonoid amount was expressed as mg quercetin equivalent (QE)/mL propolis extract. Analyzes were made in 3 replications and the results were given as the mean value.

Determination of chemical composition

The chemical composition of propolis extracts was clarified by Gas Chromatography-Mass Spectrometry (GC–MS) analysis. For this purpose, Agilent 7890A GC system HP5-MS capillary column (30 m × 0.25 mm × 0.5 mm) was used and derivatization of propolis extracts was carried out by using *N*-methyl-*N*-(trimethylsilyl)-trifluoro-acetamide (MSTFA). Shortly, propolis extracts were dried by using a rotary evaporator, and 5 mg of dried residue was mixed with 50 µL of dry pyridine and 75 µL of MSTFA. This reaction mixture was heated at 80 °C for 20 min. The oven temperature was programmed from 75 to 325 °C at a rate of 5 °C/min, and a 15 min hold at 325 °C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:50, the injector temperature was 300 °C, and the ionization voltage was 70 eV [18]. The relative ratios of the components illuminated using the library were determined.

Antioxidant activity analyses

Antioxidant activities of propolis extracts were determined by iron (III) reducing capacity test (FRAP) and DPPH radical scavenging tests.

Ferric-reducing antioxidant power (FRAP) analysis

The FRAP method is the most commonly used method for the determination of the antioxidant capacity of natural products, and it is a method based on the reduction of the iron (III) ion in the Fe(III)-TPTZ complex of antioxidant substances and hydrogen transfer [34]. Fe (III) reduced by the antioxidant substances in the solution gives an absorbance at 593 nm. Results are expressed in terms of the $FeSO_4.7H_2O$ value. Analyzes were made in 3 replications and the results were given as the mean value.

2,2-Diphenyl-1-picryhydrazyl (DPPH) analysis

The DPPH• radical (2,2-diphenyl-1-picrylhydrazil) is a commercially available radical, and a 100 µM methanolic solution of this purchased radical was used in the trials. Sample solutions at different concentrations were prepared by diluting propolis extracts with 70% ethanol. An equal volume (750 µL) of DPPH• solution and sample solutions were mixed and left at room temperature for 50 min. At the end of this period, absorbances were recorded at 517 nm, where DPPH• gave maximum absorbance. The recorded absorbance values were plotted against the concentration and the SC_{50} values were calculated. Results are expressed according to the Trolox standard [35]. Analyzes were made in 3 replications and the results were given as the mean value.

Enzyme inhibition analyses

Urease inhibition

Urease is an enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia. The amount of ammonia formed as a result of enzyme activity was determined using the indophenol method [36]. The reaction tube containing 200 µL of Jack Bean urease, 500 µL of buffer (100 mM urea, 0.01 M K_2HPO4, 1 mM EDTA and 0.01 M LiCl, pH 8.2), and 100 µL of propolis extract was incubated at room temperature for 20 min. After incubation, phenol solution (550 µL, 1% w/v phenol, and 0.005% w/v sodium nitroprusside) and alkaline mixture (650 µL, 0.5% w/v sodium hydroxide, and 0.1% v/v NaOCl) were added to the tubes and after 50 min absorbances were recorded at 625 nm. IC_{50} values were calculated using the results of enzyme activity measured using propolis extract at different amounts. Analyses were made in 3 replications and the results were given as the mean value.

Xanthine oxidase inhibition

Xanthine oxidase enzyme activity was determined by spectrophotometer measurements at 295 nm. After preincubation of 770 µL pH 7.8 phosphate buffer and 70 µL xanthine oxidase enzyme mixture at room temperature for 15 min, the reaction tube formed by adding 660 µL substrate solution was incubated at room temperature for 15 min. The reaction was stopped by adding 200 µL of 0.5N HCl. The absorbance of the reaction tubes at 295 nm was recorded [36]. IC_{50} values were calculated using the activity results obtained by adding propolis extract at different amounts to the reaction mixture. Analyzes were performed in 3 repetitions and the results were given as the mean value.

Acetylcholinesterase inhibition

The determination of acetylcholinesterase activity is based on the coloration of thiocholine with DTNB [36]. The enzyme solution was prepared in 1% gelatin solution as 2.5 Units/mL. The reaction mixture consisting of 50 µL enzyme solution, 3 mL pH 8 phosphate buffer (0.01 M), and 20 µL substrate (acetylthiocholine) solution was incubated for 5 min at room temperature. At the end of the time, 100 µL of DTNB was added to the reaction mixture and incubated for another 10 min. At the end of the relevant time, absorbance measurements were made at 412 nm and enzyme activity was determined. IC_{50} values were calculated using the activity results obtained by adding propolis extract at different amounts to the reaction mixture. Donepezil hydrochloride was used as the standard inhibitor. Analyzes were made in 3 replications and the results were given as the mean value.

Antimicrobial activity analyses

Accordingly, 20 mL of 70% ethyl alcohol was added to the 5 g sample after it was pulverized with the help of a grinder [37]. Afterward, this prepared mixture was kept in an orbital mixer (Multi Reax, Heidolph Instruments, Germany) for 30 min. The resulting solution was incubated in an ultrasonic water bath (Kudos, Shanghai, China) set at 30 °C for 45 min. This solution taken from the water bath was kept at + 4 °C for 24 h and then centrifuged at 10,000 rpm for 45 min (Hermle Z 326 K, Hermle Labortechnink, Wehngen, Germany). At the end of centrifugation, the supernatant part of the solution was taken and filtered through a 0.45 µm diameter PTFE (PolyTetraFluoroEthylene) filter. The filtered extract was kept at 60 °C for 96 h and the ethanol was evaporated. After evaporation of the solvent, the residue remaining in the tube was weighed and dimethylsulfoxide (DMSO) was added at a concentration of 100 mg/mL and vortexed. This prepared DMSO extract was kept in the refrigerator at + 4 °C until the antimicrobial activity was determined [38].

The disk diffusion method was used for the determination of the antimicrobial activity of propolis extracts. Stock cultures kept at -20 °C were inoculated into appropriate media, paying attention to aseptic conditions, and incubated at 37 °C for 24 h. During these procedures, Mueller–Hinton agar (MHA) for bacteria and Saboraud dextrose agar (SDA) for yeast-like fungi were used. At the end of the 24-h incubation period, the samples taken from single colonies were transferred to Mueller–Hinton broth and Saboraud dextrose broth media, respectively, and incubated again at 37 °C for 24 h. Microorganism suspensions obtained at the end of this process were adjusted to 0.5 McFarland standard turbidity and carefully inoculated to cover the entire surface of the media with the help of a sterile swab. After these inoculation procedures, 20 µL of the extract was impregnated on

6 mm standard discs and these discs were carefully placed in petri dishes. After these procedures, the petri dishes were incubated at 37 °C for 24 h and the inhibition zones observed around the discs at the end of the incubation period were measured with the help of a caliper and recorded.

Results and discussion

Palynological analyses

The findings obtained as a result of the palynological examination of the preparations prepared from the propolis samples obtained from the control and research group colonies are summarized in Table 1. The findings obtained in the research group propolis sample *C. sativa* L. pollen content was determined as 11.11%. The propolis sample appears to contain minor amounts of hemp pollen. *C. sativa* L. pollen is medium-sized (26–50 µm) pollen and has a triporate aperture and psilate ornamentation. The picture obtained as a result of the palynological examination of the preparations prepared from propolis samples is given in Fig. 2, 3.

As a result of palynological analyses of propolis samples, Brassicaceae and *Xanthium* spp. pollen secondary, Apiaceae, Fabaceae, Poaceae, *Echium* spp. Cupressaceae pollen minor, Asteraceae, Rosaceae, *Taraxacum* spp. pollen

Table 1 Palynological results of propolis samples

Propolis samples	Dominant pollen (> 45%)	Seconder pollen (16–45%)	Minor pollen (3–15%)	Trace pollen (< 3%)
Control group	–	Brassicaceae: 22.22 *Xanthium* spp.: 20	Apiaceae: 13.33 Fabaceae: 13.33 Poaceae: 13.33 *Echium* spp.: 6.66 Cupressaceae: 4.44	Asteraceae:2.22 Rosaceae: 2.22 *Taraxacum* spp.: 2.22
Research Group	Fabaceae: 55.55	Ranunculaceae: 22.22	Apiaceae: 11.11 *Cannabis* sativa: 11.11*	–

*As expected, cannabis was found in the research group but not in the control group

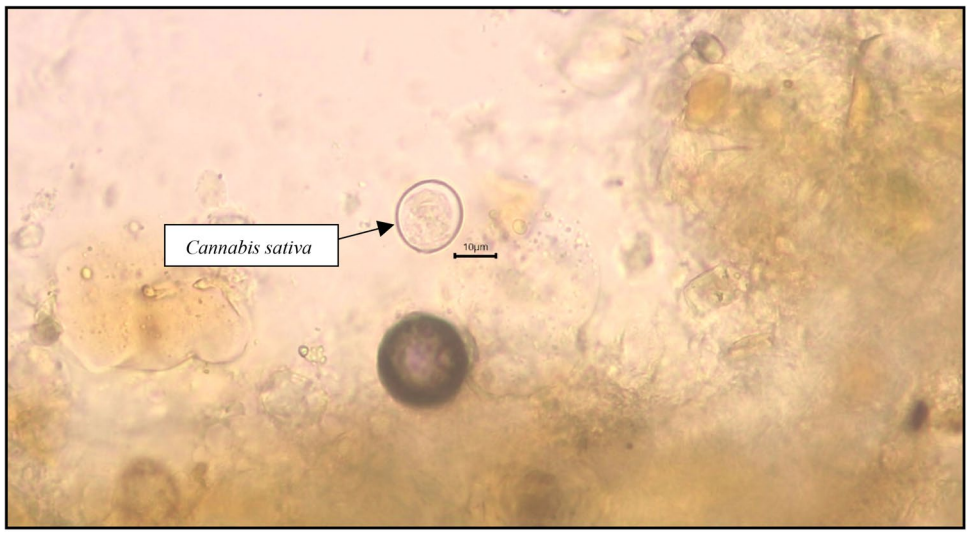

Fig. 2 *Cannabis sativa* L. pollen in propolis sample

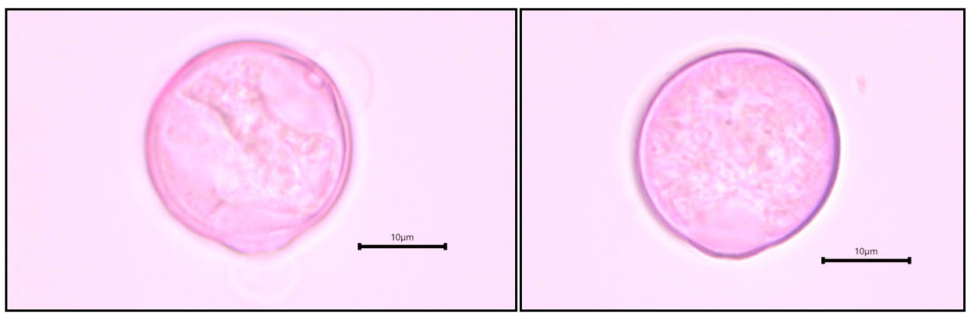

Fig. 3 *Cannabis sativa* L. pollen (×100)

trace amounts were found in the control group. On the other hand, Fabaceae pollen dominant, Ranunculaceae pollen secondary, Apiaceae, and *C. sativa* pollen minor amounts were identified in the research group samples. As expected *C. sativa* was found in the research group. Besides this, the Fabaceae family was determined at different rates in both groups, and the Apiaceae family was determined at similar rates (Table 1). The variability of the components in the two samples indicated that propolis was collected by the honey bee from different plants depending on the geographical location. Honey bees collect for propolis production according to the plant in the region and its density. In the study conducted by Kolaylı et al. [39], it was shown that the plants used by bees while producing propolis vary according to geographical and botanical origin.

In a study conducted by Gençay and Sorkun [40], 30 propolis samples collected from Erzincan province, neighboring Malatya province, were examined and 32 plant families were identified. Some species belonging to the families Apiaceae, Asteraceae, Campanulaceae, Fabaceae, Fagaceae, Lamiaceae, Liliaceae, Pinaceae, Rosaceae, Salicaceae, Rhamnaceae, Scrophulariaceae were determined as the herbal origin of propolis samples. In our study, the determination of the Apiaceae, Asteraceae, and Fabaceae families was found to be compatible with the literature report.

Propolis characterization

Dry matter, balsam, and wax amount analyses

For the physicochemical characterization of the propolis samples obtained in the study, the dry matter, balsam, and wax content were determined as % amount. The results are given in Table 2.

It was determined that the balsam value of the propolis sample obtained from the research group was higher than the control group. Keskin et al. [41] reported that the balsam values of propolis samples obtained from different provinces of Anatolia varied between 35 and 72%. In a study on propolis standardization, it was reported that the balsam ratios of propolis samples obtained from different regions of Turkey ranged from 23.6 to 71.1% [29]. In the study in which twenty different propolis extracts were used, it was found that the brix value was between 25 and 61 for ethanolic propolis extracts and the balsam values were between 7.1 and 95% [42]. In a study by Keskin et al. [43] examining Bilecik and Malatya propolis, it was reported that the amount of balsam varied between 10.3 and 40.6%. In a study examining Bilecik propolis [44], it was reported that the amount of balsam varied between 13 and 52%. Pavlovic et al. [45] in which hill and plain-type propolis samples were examined, the amount of balsam in the hill-type samples (75.92%) was found to be higher than the amount of balsam (63.94%) of the plain-type samples.

In a study conducted with propolis and honeycomb wax samples collected from hives, it was stated that the wax content in propolis samples was between 11.2 and 29.3%. In both types of wax, monoesters formed the largest fractions (62.1–86.6%). Monoesters were followed by hydrocarbons (6.9–24.7%) [46]. Negri et al. [47] reported the wax content of ethanol extracts of propolis samples collected from Brazil and Uruguay between 2.3 and 16.4%. In a study conducted by Keskin and Kolaylı [29], the wax amount of different Turkish propolis was determined. According to this study, it was stated that the amount of wax in Turkish propolis was $5 \pm 2.8\%$ approximately. In this respect, the data obtained in the study was compatible with the literature.

Balsam value, which is defined as the fraction dissolved in ethanol, is an important quality parameter for raw propolis samples. Because the high balsam value shows high biological activity. The balsam value of the hemp propolis sample obtained from the research group is higher than the control group sample, revealing the importance of the cannabis plant.

Table 2 Dry matter, balsam, and wax amount results of propolis samples

Parameter	Control group	Research group
Dry matter (%)	93.8 ± 1.4^{a}	94.6 ± 1.8^{a}
Balsam quantity (%)	40.9 ± 5.9^{b}	46.2 ± 5.2^{c}
Wax amount (%)	4.5 ± 0.8^{d}	4.8 ± 0.5^{d}

*Different letters in the same columns show statistical differences between means ($p < 0.05$)

The total amount of phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activity analyses

For the biochemical characterization of propolis samples, total phenolic substance, total flavonoid substance, and antioxidant activity determinations were carried out. The obtained results are given in Table 3. It was determined that the total amount of phenolic substance in the research group propolis sample was higher than the control group. The antioxidant capacity of the research group propolis sample was also found to be high depending on the total amount of phenolic substances.

An important parameter for propolis extracts is the total amount of phenolic substances. Keskin and Kolaylı [29], determined the total phenolic content of Anatolian propolis was between 16.13–178.34 mg GAE/g for raw propolis, Keskin et al. [43] reported the total phenolic content of propolis was between 5.42 and 35.46 mg GAE/mL and the maximum flavonoid content found as 4.69 mg QE/mL. Keskin et al.

Table 3 The total amount of phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activity analyses

	Total phenolic content (mg GAE/mL)	Total flavonoid content (mg QE/mL)	FRAP (μM $FeSO_4 \cdot 7H_2O$/mL)	DPPH SC_{50} (mg/mL)
Control group	23.18 ± 0.92	1.98 ± 0.07	87.08 ± 2.35	37.21 ± 1.52
Research group	26.44 ± 1.08	2.03 ± 0.11	88.16 ± 2.27	33.08 ± 1.45

[41] emphasized that the total phenolic content of Anatolian propolis samples ranged from 28 mg GAE/mL to 80 mg GAE/mL. In another study performed by Kurek-Górecka et al. [48], it was reported that the total phenolic content of Turkish, Poland, Uruguay, and Romanian propolis samples varied between 85.328 and 155.279 mg/g dry extract. When the findings were evaluated with the literature data, it is seen that the propolis samples obtained are in the range of Anatolian propolis samples in terms of total phenolic substance and antioxidant capacity.

The beneficial biological properties of propolis are mostly attributed to phenolic substances such as flavonoids and hydroxycinnamic acid derivatives. Flavonoids are strong antioxidant, radical scavenging polyphenolic conjugated aromatic compounds with different chemical structures and characteristics [49]. Kitamura et al. [50] emphasized that Brazilian propolis is especially rich in flavonoids and cinnamic acid derivatives. Keskin et al. [44] reported that the total phenolic content of Bilecik propolis ranged from 11 mg GAE/mL to 76 mg GAE/mL, and the total amount of flavonoids ranged from 2.66 mg QE/mL to 16.47 mg QE/mL. Woisky and Salatino [13] reported the total polyphenol content of Brazilian propolis between 8.8 and 13.7% and the flavonoid content between 0.35 and 2.7%.

Özkök et al. [51] analyzed 23 propolis samples to determine propolis' standard antioxidant content and compounds. They found the total flavonoid content between 21.28 and 152.56 mg QE/g, and the total phenolic content between 34.53 mg and 259.4 mg GAE/g. They also found the CUPRAC antioxidant capacity range of propolis samples between 95.35 and 710.43 mg TE/g. In their study, Keskin and Kolaylı [42] examined 20 different propolis extracts and found the total amount of phenolic substances between 1 and 95% and the content of total flavonoid substances between 0.1 and 7.8%. Pavlovic et al. [45] 's study showed that the total phenols of the hill and plain propolis samples were 242.42 mg GAE/g and 236.32 mg GAE/g, and their total flavones and flavonols were 32.14 mg QE/g with 26.91 mg QE/g and DPPH radical scavenging activity was 45.01% with 46.44% determined. In 5 propolis samples collected from Slovakia and the Czech Republic, flavanones/dihydroflavonols were found to be 3.63–6.24%, and flavones/flavonols were found to be 5.88–9.9% [52]. Southeastern Brazilian Bee propolis samples contained 48.46 mg/g, and Southern Brazilian Bee propolis samples contained 23.7 mg/g flavonoids [53]. Moreno et al. [54] reported that propolis samples contain high rates (13.3–42.6 mg/g propolis) of flavonoids. Mohamed et al. [55] reported DPPH EC_{50} value of propolis extract as 1.78 mg/mL, the total phenolic substance amount as 31.99 mg GAE/g, and the total flavonoid substance amount as 66.4 mg QE/g. The biochemical activity of propolis depends on many factors such as botanical origin, and propolis production technique. When the findings were compared with the literature, it was clear that the data obtained in the study was compatible with the literature.

Chemical composition of propolis samples

Chemical compounds of propolis samples were determined by GC–MS device and are given in Table 4 as % area.

Table 4 Chemical composition of propolis samples

Chemical compound	Control group	Research group
Ferulic acid	0.82	1.98
Caffeic acid	2.63	2.86
Benzoic acid	0.16	0.18
Hydrocinnamic acid	0.12	1.08
4-Hydroxy,3-methoxy cinnamic acid	0.76	0.81
p-Methoxy cinnamic acid	0.26	0.23
3-Methyl-2-butenyl isoferulate	0.02	0.02
3,5,7-Trihydroxy flavone	–	0.35
Linalool oxide	0.05	0.06
Succinic acid	1.98	1.46
Malic acid	0.41	–
Palmitic acid	0.69	0.72
Stearic acid	0.15	0.17
Oleic acid	0.15	0.38
Oktadecanoic acid	0.03	0.04
Fructose	0.13	0.30
Sorbose	1.96	0.18
D-Galactose	0.04	0.03
Mannose	0.45	1.18
Cannabidivarinic acid	–	0.29
Delta (1)-Tetrahydrocannabinolic acid	–	0.59
Cannabidiolic acid	–	0.48

Propolis has a rich composition and contains bioactive compounds such as phenolic acids, flavonoids, and terpenes [56]. When Table 4 is examined, it is seen that propolis samples are rich in phenolic acids and cinnamic acid derivatives. Also, it was determined that the research group propolis sample contains cannabinoid derivatives (cannabidivarinic acid, 0.29%; delta (1)-tetrahydrocannabinolic acid, 0.59%; cannabidiolic acid, 0.48%), which are active compounds of the cannabis plant. Accordingly, we can say that the variability of the components in the propolis content varies depending on the plants from which the honey bees collect the propolis. The content of propolis varies depending on the method and time of collection, bee breed, and the flora of the region where it is collected [42].

Keskin et al. [44] stated in their study that Bilecik propolis samples are rich in aldehydes, aliphatic acid and esters, alcohols, hydrocarbons, carboxylic acid esters, ketones, terpenes, fatty acids, and other components. According to Bankova et al. [57] determined that the propolis samples collected monthly from African and European *A. mellifera* colonies were phenolic compounds, especially cinnamic acid derivatives, as the main compounds.

According to Tazawa et al. [58] isolated 7 new p-coumaric acid derivatives with 17 known compounds including 4 flavonoids, 1 phenolic acid, 4 diterpenoic acid, 1 lignan, 2 p-coumaric acid esters and 5 cinnamic acid derivatives from 75% ethanol extract of Brazilian propolis. Five compounds [dihydrocampferol (aromadendrin), 6-methoxycampferol, 4-hydroxy-3-prenyl benzoic acid, plicatin B, and capillarthemycin A] were isolated from propolis for the first time.

Kolankaya et al. [59] reported that Turkish chestnut (*Castanea sativa*) propolis mainly contains important flavonoids such as galangin, quercetin, kaempferol, apigenin, pinobanksin, pinocembrin and pinostrobin, and these flavonoids are very important in herbal medicine. Mohamed et al. [55] identified 17 compounds including terpene and polyphenol in propolis extract obtained from Malaysian stingless bees (*Tetrigona apicalis*).

Cannabis has been used in traditional medicine around the world for thousands of years. However, not all of its active ingredients and mechanisms of action have yet been discovered. Naturally, the effect of cannabis is mostly based on cannabinoids [7]. While honey bees carry cannabis pollen to the hive, they also carry the active compounds of the cannabis plant [60]. There is no data in the literature on the chemical composition of propolis obtained from the cannabis plant [26], which predominantly contains components such as cannabidiol, cannabidiolic acid, cannabigerol, and cannabigerolic acid. The components we obtained from the research group propolis in our study may contribute to the literature in terms of describing the composition of cannabis propolis.

Enzyme inhibition analyses

The inhibition effects of ethanol extracts prepared from the obtained propolis samples on urease, xanthine oxidase, and acetylcholinesterase enzyme activities were examined and the obtained results are given in Table 5. When the IC_{50} values, which are an indicator of the inhibitory effect of propolis samples on the related enzymes, are examined, it is seen that there is no significant difference between the propolis groups, but the related enzymes have lower values than the standard inhibitors. In general, a small IC_{50} value is expressed as a high inhibitory effect.

In a study, the inhibition effects of propolis samples obtained from different provinces of Anatolia on urease, xanthine oxidase, and acetylcholinesterase enzymes were investigated. In the study findings, it was stated that all propolis extracts exhibited inhibitory effects on these enzymes with variable IC_{50} values in the range of 0.074–1.560 mg/mL, and propolis extract with high phenolic content also had a high inhibitory effect. The IC_{50} values of the propolis sample obtained from the province of Zonguldak with the highest phenolic content for the mentioned enzymes were reported as 0.081 ± 0.009, 0.080 ± 0.006, and 0.074 ± 0.011 µg/mL, respectively [36].

The correlation between obtained data was presented in a heat map below (Table 6).

Antimicrobial activity analyses

The antimicrobial activity of propolis samples against some pathogenic gram-positive (6 units), gram-negative

Table 5 Inhibition effects of propolis extracts on some enzymes

	Urease IC_{50} (mg/mL)	Xanthine oxidase IC_{50} (mg/mL)	Acetylcholinesterase IC_{50} (mg/mL)
Control group	0.31 ± 0.01	0.43 ± 0.02	0.77 ± 0.01
Research group	0.28 ± 0.01	0.37 ± 0.02	0.72 ± 0.01
Acetohydroxamic acid	18.42 ± 0.14	–	–
Allopurinol	–	0.74 ± 0.05	–
Donepezil	–	–	12.18 ± 0.09

Table 6 Heat map of biochemical activity of research and control groups

	Cannabis sativa L.	Total Phenolic Content	Total Flavonoid Content	FRAP	DPPH	Urease Inhibition	Xanthine Oxidase Inhibition	Acetylcholinesterase Inhibition
Control Group	-	23.18	1.98	87.08	37.21	0.31	0.43	0.77
Research Group	11.11	26.44	2.03	88.16	33.08	0.28	0.37	0.72

(6 units), and fungi (7 units) microorganisms was determined by the disc diffusion method. The results are given in Table 7. It can be seen from the table that the control group and research group propolis samples had higher zone diameters against gram-positive microorganisms, but there was no difference between the groups. Likewise, it is understood from the table data that propolis samples obtained from both groups have high antimicrobial activity against the tested fungi, but there is no significant difference between the groups.

In this study, samples were extracted by ultrasonic extraction method during the determination of antimicrobial activity. During the ultrasonic extraction processes, ethanol was used as a solvent and the samples were kept in a Pasteur oven at 60 °C for 96 h in order to evaporate the solvent from the prepared extract. The residues obtained were weighed and DMSO was added to them and the concentration was adjusted to 100 mg/mL. The disk diffusion method was used to determine the antimicrobial activities of propolis samples.

The results obtained in antimicrobial activity tests showed that propolis samples had an inhibitory effect against all fungi samples. The highest inhibition effect of propolis samples in fungi was observed in *Candida albicans* EA1 strain and inhibition zone diameters were measured as 14–15 mm. *Candida glabrata* EA38 strain was found to be the least sensitive fungi sample against propolis samples, and the zone diameters were measured as 11 mm for both extracts. When the results obtained were compared with the literature, it was seen that the results were compatible, but the MIC and MBC values were found quite high [61–65]. When evaluated in terms of bacteria, it was seen that propolis samples formed fewer inhibition zones in bacteria compared to fungi. The highest inhibition zone diameter (13 mm) in bacteria was measured in *Enterococcus faecalis* NCTC 12697 strain.

Table 7 Antimicrobial activity results of propolis samples

Microorganisms	Positive cont		Control group			Research group		
	AMP	FLU	ZONE	MIC	MBC	ZONE	MIC	MBC
Gram-positive								
B. cereus BC 6830	33	–	12	12.5	50	11	25	50
E. faecalis NCTC 12697	31	–	13	12.5	25	13	12.5	50
E. faecalis ATCC 49452	29	–	9	25	50	9	25	50
S. mutans ATCC 35668	30	–	8	25	50	8	25	50
S. aureus NCTC 10788	31	–	8	25	50	7	25	50
S. aureus ATCC 25923	27	–	7	50	–	7	50	50
Gram-negative								
A. baumannii ATCC 19606	14	–	–	50	–	7	50	50
E. coli NCTC 9001	12	–	8	25	50	7	50	–
E. coli ATCC 25922	–	–	8	25	50	7	50	50
P. aeruginosa NCTC12924	9	–	–	50	–	–	–	–
S. typhimurium RSSK95091	13	–	8	50	50	9	50	50
Y. enterocolitica ATCC 27729	11	–	8	50	50	8	50	50
Fungi								
Candida albicans EA1	–	30	14	6.25	12.5	15	6.25	12.5
Candida glabrata EA5	–	28	12	12.5	25	12	12.5	25
Candida krusei EA8	–	24	11	12.5	25	12	12.5	50
Candida glabrata EA38	–	27	11	25	25	11	25	25
Candida albicans EA60	–	29	13	12.5	25	12	12.5	25
Candida albicans ATCC10231	–	26	12	12.5	25	12	12.5	25
Saccharomyces cerevisiae SB1	–	31	13	12.5	25	13	12.5	25

ZONE inhibition zone diameter: mm, *MIC* minimum inhibition concentration mg/mL, *MBK* minimum bactericidal concentration mg/mL, *AMP* ampicillin, *FLU* fluconazole

In addition, it was observed that *Pseudomonas aeruginosa* NCTC 12924 strain was resistant to propolis samples and no inhibition zone was formed. In addition, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NCTC 9001, and *E. coli* ATCC 25922 strains were observed to be less sensitive to propolis samples compared to other bacteria. When we make a comparison in terms of Gram-positive and Gram-negative bacteria, it is concluded that Gram-positive bacteria are more sensitive to propolis samples than Gram-negative bacteria. It is seen that these results are compatible with the literature [66–70]. Yasmeen et al. [71] reported that *C. sativa* exhibited activity both against Gram-positive and Gram-negative bacteria and also against fungi. In our study, it can be thought that the difference between the control group and the research group is due to the cannabis plant.

Conclusion

In this study, palynological analyses showed that honey bees prefer cannabis plants when collecting propolis, and the dry matter percentage, balsam and wax content, total phenolic and total flavonoid content, and antioxidant capacity of the research group propolis samples containing hemp were found to be higher than the control group. Also, a high inhibition effect of cannabis propolis (research group) was detected on some medically important enzymes such as urease, xanthine oxidase, and acetylcholine. In addition, the research group propolis samples also showed a high antimicrobial effect against many pathogenic microorganisms. This study is the first study on cannabis propolis and the results will be data for the use of cannabis propolis in apitherapy applications or other different fields.

Acknowledgements This study was supported by The Scientific and Technological Research Council of Türkiye (TUBITAK) with Project number 120O845.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This study does not contain any studies with human participants or animals performed by the author.

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