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Comparison of fatty acid properties of Bingol Propolis

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Abstract

The purpose of this study was to determine the chemical and physical properties of samples of propolis collected from apiaries in Genç, Karlıova and Solhan districts of Bingöl Province, which is important in the beekeeping sector in Turkey. In the experiment, fatty acids and derivatives of the propolis samples were analysed with gas chromatography-mass spectrometry (GC/MS) analysis techniques. As a result, 10 fatty acids and 49 volatile components were detected in these samples. These results will contribute to the standardization of propolis produced in Bingöl, Turkey, and to future studies on determining the chemical composition of propolis.

Keywords: fatty acids, honey bees, volatile components
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Introduction

Honey bees have existed for millions of years. In the process of evolution they have built up mechanisms for survival, even with changing environmental challenges (Lazarov & Zhelyazkova, 2020). The success of honey bees in the evolutionary process enabled them to spread to almost all habitats on earth and to live for many years. In Turkey, beekeeping was practised as a traditional agricultural activity because of its contribution to agricultural production and its importance in human nutrition and health. Honey bees (*Apis mellifera L*.) have become one of the most important creatures of the ecosystem because they create valuable healthy products throughout their life cycle and contribute to people's quality of life (Popova *et al.,* 2004; Yücel 2015). Propolis is one of these products.

Honey bees collect nectar and pollen from plants (Shumkova *et al.,* 2020). Propolis is a mixture of beeswax and substances collected by honeybees from buds, branches, leaves of trees and some herbaceous plants. It is a sticky, resinous fragrant substance, with colours from dark yellow to brown, and is used for many purposes in the hive (Borba *et al.,* 2015).The word 'propolis' comes from ancient Greek *pro* ('first' or 'defence') and *polis* ('city'), and was used by the Greeks to mean 'front defence'. Propolis was widely used as a medicine by the Egyptians, Greeks, and Romans in ancient times. The Egyptians used it to treat some diseases and to mummify the dead. The Greeks and Romans also used it to heal skin abscesses. Propolis, which was praised by Hippocrates, Herodotus, Aristotle, and other ancient philosophers, has been used since ancient times to treat certain diseases or to ameliorate their effects (Castaldo & Capasso, 2002). It is generally accepted that bees collect propolis from resinous tree buds and many trees have been proposed as its source (Crane, 1988). However, only a few chemical analyses have been performed to confirm these proposals.

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Bees produce propolis by mixing pollen, resin, and waxy substances from plants with active enzymes secreted from glands between their head and thorax (Ghisalberti, 1979; Marcucci *et al.,* 2001). Worker or field bees can carry an average of 10 mg of propolis. They break down the sap or resin of the plant, carry it to the hive with the corbicula on the tibia of the third hind leg. Worker bees in the hive combine propolis with enzymes, pollen and beeswax in their mouths, and produce the final form (Sımoes-Ambrosıo *et al.,* 2010).

Honeybees produce propolis for various purposes, such as repairing holes and cracks in the hive, narrowing the entrance or isolating the hive from the outside environment, mummifying harmful organisms entering the hive, and protecting the colony from disease (Kumova *et al.,* 2002). Thanks to its chemical properties, propolis helps to balance air circulation in the hive and prevent the development of harmful microorganisms (Ghisalberti, 1979; Kumova *et al.,* 2002). The resin that is used in the production of propolis is produced by plants as a defence mechanism against pathogens (Giada 2013).

Propolis is regarded as a health-promoting food with many therapeutic (antibacterial, antifungal and antioxidant) activities (Kahraman *et al.,* 2022). More than 300 components have been reported to exist in the chemical content of propolis, but only 180 have been identified. Propolis generally contains 50% resin, 30% wax, 10% oil, 5% pollen, and 5% vitamins, minerals and simple sugars (Burdock, 1998). Because of its biological activities, propolis can contribute to the development of new drugs for use in human and veterinary health (Sforcın & Bankova, 2011), apitherapy, and cosmetics (Graikou *et al.,* 2016; Santos *et al.,* 2020; Farag *et al.,* 2021; Mutlu *et al.,* 2022). Propolis is used today in the production of yoghurt, fruit juice, cream, toothpaste, lotion, tea, etc.

The chemical composition of propolis varies according to the ecological characteristics of the area in which it was collected, such as vegetation and climate. This contributes to its diversity, but prevents propolis from chemical standardization and poses a problem for quality control (Kumova *et al.,* 2002; Bankova, 2005). The differences in propolis content are caused by the preferences of the bees and the plant resources in the region in which the colony is located (Bankova, 2016).

Propolis has multiple biological and pharmacological properties including the regulation of energy homeostasis (Fuliang *et al.,* 2005; Kitamura *et al.,* 2013; Nakajima *et al.,* 2014). However, the mechanisms underlying it have not been fully elucidated. More than 300 compounds have been found in propolis, which include phenolic acids, cinnamic acids, caffeic acids and their esters, flavonoids, terpenes, aromatic aldehydes and alcohols, fatty acids, stilbenes, and steroids (Akyol *et al.,* 2013; Li *et al.,* 2016).

Beekeepers must know the plant sources of propolis in the region. If the bees cannot find suitable plant sources in their environment, they may use unwanted substances, such as paint, pitch, asphalt, and mineral oils as replacements, which could cause problems in the pharmacological and medical uses of propolis (Bankova *et al.,* 1995). The aim of this study was to contribute to the literature on the standardization of propolis in Turkey, especially in Bingöl, and to determine the chemical composition of propolis in this region. The quality of propolis in Bingöl was compared with reports from the literature.

Material and Methods

Propolis samples were procured from the active beekeepers who were affiliated to the Bingöl Beekeepers Association. Bingöl is located in the Upper Euphrates section of Eastern Anatolia between the east longitudes of 38°27' and 40°27' and the north latitudes of 41°20' and 39°54'. Bingöl is bordered by Muş to the east, Erzincan and Erzurum to the north, Tunceli and Elazığ to the west, and Diyarbakır to the south. Bingöl is rich in forests, especially oak. These forests spread up to the altitude of 1900 m. The total area of the province is 812,537 hectares. Of this area, 7.28% is used as agricultural land, 27.92% as forest, 10.25% is becoming forested, 51% as pasture, 2.2% as meadow, and 1.3% as other.

The propolis samples were collected in November and December 2019 from the hives in the apiaries in Yaz Konağı village, Sağgöze village and Çotla plateau in Genç; Kaynarpınar village, Halifan village and Kargapazarı village in Karlıova; and Şerafettin plateau, Bozkanat village and Göksu village in Solhan (Table 1). Three propolis samples were collected from each apiary to represent the population of the hives. The samples were scraped with a spatula from the hive entrances and exits, hive bottom board, flight holes, and spaces between the hive covers. After labelling, the samples were kept frozen until analysed.

Table 1 Regions and altitudes of villages where propolis samples were collected

Genç	Altitude, m	Karlıova	Altitude, m	Solhan	Altitude, m
Yaz Konağı village	1645	Kaynarpınar village	1630	Şerafettin plateau	2544
Sağgöze village	1723	Halifan village	1797	Bozkanat village	1915
Cotla plateau	2346	Kargapazarı village	1672	Göksu village	1725

Hara & Radin's (1978) method was revised and used for lipid extraction. About 2 g of the frozen raw propolis samples were ground in a powerful grinder to increase the surface area. The samples were fragmented in 10 mL hexane/isopropanol (3:2) for 30 seconds at 10 000 rpm in a homogenizer, and centrifuged at 5 000 rpm for 10 minutes. The top portion was taken, filtered, and put in test tubes. Derivatization is needed for fatty acids to be looked at in gas chromatography. Methyl esters are preferred for derivatization. For this purpose, Christie's (1990) method was used because it is practical and highly efficient. The prepared lipid extract was placed in 30 mL capped tubesaAnd 5 mL 2% methanolic sulfuric acid was added and vortexed. This mixture was left to methylate for 15 hours in an oven at 50 °C. Then the tubes were cooled to room temperature, and 5 mL of 5% sodium chloride was added and vortexed. Fatty acid methyl esters that formed in the tubes were extracted with 5 mL hexane, and the hexane phase was taken from the top with a Pasteur pipette and treated with 5 mL of 2% sodium bicarbonate. It was left for 1-2 hours to separate the phases. Then, the solvent of the mixture containing methyl esters was evaporated under nitrogen at 45 °C, and the fatty acids at the bottom of the test tubes were dissolved with 1 mL hexane, taken into GC vials, and analysed in a GC-MS device.

Volatile compounds and fatty acids in the propolis samples were determined by gas chromatography using the Agilent 7890A/5970 C GC-MS (Santa Clara, California, United States) with a 100m x 0.25 mm x 0.25 um capillary column. The temperature was increased gradually from 120 °C to 250 °C, and the total time was set as 40 min. The sample was heated at a rate of 5 °C/min and retained for 14 minutes at this temperature. The total time was 40 minutes. The autosampler rinsed itself five times with hexane before taking the sample, and then the sample was delivered to the column. The injection volume was 1 µL and split ratio was 25:1, solvent delay time was 12 minutes. The carrier gas was helium, and its flow was 1 mL/min when constant gas flow was set. The hydrogen flow was 35 mL/min. The dry air flow rate was 350 mL/min. The nitrogen flow rate was set automatically at 20.227 mL/min by the program. The results were evaluated by comparing them with the data in the Wiley and NIST libraries in the device's memory.

The SPSS 11.0 software for Windows (IBM Corp., Armonk, New York, USA) was used for statistical calculations. Arithmetic means and standard deviations were calculated. Differences between groups were evaluated by one-way analysis of variance with the threshold $P \leq 0.05$ indicating significant differences. Mean separation was accomplished using Duncan's test.

Results and Discussion

Fifty-nine compounds were identified in the samples of propolis. Of these compounds, 10 were fatty acids and 49 were volatile components. Even the samples from the same region were heterogeneous. Table 2 shows the saturated fatty acid (SFA) contents of the samples from Genç, Karlıova, and Solhan regions. No significant differences between these villages were detected for total SFA and the contents of capric, lauric, arachidic, and behenic acids. However, a lower amount of myristic acid and higher amounts of palmitic acid and lignoceric acid were found in the samples from Sohan compared with those from Genç and Karlıova. Arachidic acid and behenic acid were observed in the propolis samples from Genç and Karlıova, but not in those from the Solhan region

Table 2 Saturated fatty acid content of propolis obtained from three regions in Bingöl province

Fatty acids	n	Retention time	Genç	Karlıova	Solhan	Significance
Capric acid (C10:0)	3	28.217	0.08 ± 0.02[a]	0.09 ± 0.03[a]	0.07 ± 0.01[a]	*P* <0.05
Lauric acid (C12:0)	3	33.247	0.80 ± 0.39[a]	0.45 ± 0.02[a]	0.43 ± 0.16[a]	*P* <0.05
Myristic acid (C14:0)	3	38.682	1.40 ± 0.27[a]	1.39 ± 0.36[a]	0.72 ± 0.21[b]	*P* <0.05
Palmitic acid (C16:0)	3	44.084	16.08 ± 0.63[a]	15.07 ± 1.05[a]	20.97 ± 2.25[b]	*P* <0.05
Arachidic acid (C20:0)	3	52.987	3.75 ± 1.89[a]	3.45 ± 1.78[a]		*P* >0.05
Behenic acid (C22:0)	3	56.621	2.96 ± 0.93[a]	2.16 ± 1.17[a]		*P* >0.05
Lignoceric acid (C24:0)	3	59.745	8.13 ± 0.39[a]	9.09 ± 0.82[a]	11.53 ± 1.21[b]	*P* <0.05
Total SFA,%	3	-	33.2 ± 4.52[a]	31.7 ± 5.23[a]	33.72 ± 3.84[a]	*P* >0.05

[a,b] Within a row, means with a common superscript were not different with probability *P* =0.05
n: Number of samples assayed, SFA: saturated fatty acid

Table 3 shows the unsaturated fatty acid content of samples from Genc, Karlıova and Solhan regions. Although there were no significant differences between Genç, Karlıova and Solhan in the amounts of oleic acid, linoleic acid, and total monounsaturated fatty acid, the amount of linolenic acid varied between the regions. As a consequence, the total polyunsaturated fatty acid was higher in samples from Solhan than from the other regions. Diğrak *et al.* (1995) identified eight fatty acids in propolis samples from Elazig, namely capric (0.74%), pentadecanoic (0.30%), palmitic (9.6%), stearic (2.72%), linoleic (0.42%), arachidonic (1.26%), eicosapentaoneic (2.60%), and nervonic acid (15.8%). Shakya *et al.* (2018) identified these fatty acids in propolis produced by bees in Jordan: palmitic (44.5%), arachidic (7.4%), steraic (5.4%), linoleic (3.1%), caprylic (2.9%), lignoceric (2.6%), cis-11,14-eicosadienoic (2.4%), palmitoleic (1.5%), cis-11-eicosenoic (1.2%), α–linolenic (1.1%), and cis-13,16-docosadienoic (1.0%). Şahinler & Kaftanoğlu (2005) reported that propolis contained caprinic acid (0.02%) and lauric acid (0.33%). Koru *et al.* (2007) recorded that the palmitic acid content of propolis was (0.33–2.39%) and that it contained 0.11–1.23% stearic acid as well. Koru *et al.* (2007) reported that propolis contained oleic acid (0.63–1.00%), α-linoleic acid (0.4%), 9-Octadecanoic acid (0.36–2.12%). Finally, Silici & Kutluca (2005) recorded the content of behenic acid as 1.02–1.88%. The diversity in these findings points to the problem of lack of consensus of the fatty acid composition of propolis.

Table 3 Unsaturated fatty acid content of propolis obtained from three regions in Bingöl province

Fatty acids	n	Retention time	Genç	Karlıova	Solhan	Significance
Oleic acid (C18:1)	3	49.217	16.77 ± 2.5[a]	19.16 ± 2.99[a]	18.76 ± 0.90[a]	*P* >0.05
Linoleic acid (C18:2)	3	50.206	1.40 ± 0.78[a]	1.92 ± 0.21[a]	1.62 ± 0.27[a]	*P* >0.05
Linolenic acid (C18:3)	3	51.620	0.54 ± 0.31[a]	1.14 ± 0.12[b]	0.86 ± 0.06[c]	*P* <0.05
Total MUFA,%	3		16.77 ± 2.50[a]	9.16 ± 2.99[a]	18.76 ± 0.90[a]	*P* >0.05
Total PUFA,%	3		1.94 ± 1.09[a]	3.06 ± 0.33[a]	2.48 ± 0.33[b]	*P* < 0.01

[a,b,c] Within a row, means with a common superscript were not different with probability *P* =0.05
n: Number of samples assayed, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

Gas chromatography was used to identify 49 aromatic acids, phenolic acids, fatty acids and esters; aldehydes, carboxylic acids and hydrocarbons; terpenes and terpenoids; and alcohols and ketones contained in propolis. The numbers and proportions of these compounds varied from region to region. Eighteen compounds were found in the samples from all three regions (Table 4). Unfortunately, the compounds that were commonly found in samples from across Bingöl represented approximately one half of the chemical content of propolis that could be detected by gas chromatography. The common compounds were generally similar to those reported in earlier studies of the chemical composition of propolis with gas chromatography.

Table 4 Compounds detected by gas chromatography that were common to all three regions in Bingöl Province where the propolis samples were collected

Aromatic, phenolic, fatty acids and esters	Retention time	Genç (%)	Karlıova (%)	Solhan (%)
2-Propenoic acid	65.118	0.26	1.20	1.52
Methyl cinnamate	46.093	0.89	0.77	0.58
Acetic acid	19.371	0.31	0.21	0.43
Aldehydes, carboxylic acids and hydrocarbons				
Eicosane	31.622	0.06	0.12	0.16
Docosane	37.115	0.44	0.38	0.41
Tetracosane	19.359	0.55	0.7	0.86
Trionsane	57.262	4.20	4.1	4.82
Pentacosane	25.516	1.62	1.8	2.01
Benzaldehyde	39.621	0.28	0.29	0.17
Undecane	18.287	0.28	0.27	0.29
Tetradecane	19.394	0.45	0.4	0.42
Octadecane	37.057	0.19	0.29	0.51
Nonadecane	23.994	0.67	0.93	0.65
Decane	24.012	1.55	1.38	1.38
Isopropyl hexadeconoate	19.365	1.20	1.35	1.38
17-Pentatriacontene	66.640	6.67	5.24	5.08
Terpenes and terpenoids				
Eudesmol alpha	46.825	0.13	0.42	0.24
Alcohols and ketones				
Phenol	45.320	1.19	1.07	1.34

Table 5 shows the remaining – more regionally specific – compounds. In Gülgen's (2016) master's thesis, it was reported that α-bisabolol (9.44%), β-caryophyllene (7.77%), tetradecanoic acid (7.66%), farnesyl acetone (7.17%), and heptadecane (3.28%) were abundant in the essential oil extract of propolis from Bingöl. These compounds were not found in the propolis that was analysed in the current study. Melliou *et al.* (2007) identified 94 components of essential oil extracted from Greek propolis. Among these were junipen, α-pinene, manoyl oxide, trans-β-terpineol, α-eudesmol, n-decanal, guaiol, δ-kadinen, α-muurolene, and cedrol. The percentages of these components differed depending on the region from which the propolis was collected. In their study on the essential oil extracts of Brazilian propolis, Ioshida *et al.* (2010) also reported the presence of α-pinene (18.3%). Popova *et al.* (2005) reported that the propolis samples from Artvin contained high amounts of 9-octadecenoic acid, myristic acid, hexadacanoic, benzoic acid, and heptadecane. Those from Bursa were high in myristic acid and benzoic acid. Those from Hatay were high in 9-octadecenoic acid, benzoic acid, benzyl cinnamate, and octadecane. Those from Kayseri were high in hexadecanoic acid and benzoic acid. Those from Yozgat were high in benzoic acid. Those from İzmir were high in benzoic acid and myristic acid, and those from Adana were high in myristic acid.

When the results of these studies were compared with the present study, Bingöl propolis was found to show that despite some similarities, the chemical compounds in propolis samples varied among regions. These differences might result because the regions differed in climate and vegetation. Thus, it is not surprising that propolis samples collected from different regions varied in their chemical composition depending on the plant sources, collection time period, and collection techniques that characterize the individual samples (Uzel *et al.,* 2005; Bankova *et al.* 2014; Oruç *et al.,* 2017).

Table 5 Compounds detected by gas chromatography that had some degree of regional specificity in Bingöl Province where the propolis samples were collected

Aromatic, phenolic, fatty acids and esters	Retention time	Genç (%)	Karlıova (%)	Solhan (%)
Benzoic acid	31.782	0.38	-	-
Hexacosanoic acid	63.007	3.28	-	4.90
Hexadecanoic acid	25.553	5.87	5.96	-
Benzeneprepanoic acid	-	-	0.25	-
12-Octadecanoic acid	68.208	0.2	5.85	-
Azelaic acid	47.077	-	0.16	-
Naphtalene carboxylic acid	47.054	1.15	-	-
Methyl cinnamate	46.093	0.89	0.77	0.58
Acetic acid	19.371	0.31	0.21	0.43
Benzyl cinnemate	67.973	1.82	-	-
Methylp-methoxycinnamete	31.235	-	-	1.27
9-Methoxy bicylo	37.910	-	-	0.79
6-Trimethyl-4H-cyclopent	57.782	-	-	5.68
Aldehydes, carboxylic acids and hydrocarbons				
Heneicosane	18.267	1.58	1.13	-
Nonacosane	60.758	4.27	-	-
9-Tricosane	65.152	1.1	-	0.17
4-Propyl benzaldehyde	39.661	-	0.24	0.17
Propyl benzaldehyde	39.627	0.35	-	-
Undecane	18.287	0.28	0.27	0.29
Pentadecane	31.135	0.38	-	0.60
Hexadecane	31.198	0.05	0.36	-
Tridecane	34.139	0.12	0.15	-
Tetratriacontone	41.286	-	0.02	-
6-dimethyl decane	13.683	-	0.12	-
2-Hydroxy cyclopenta decanone	66.543	-	-	5.56
Cholestan-3-one	69.804	-	0.08	-
Pyridine-3- carboxamide	46.842	-	-	0.1
Terpenes and terpenoids				
Eudesmol beta	47.437	-	0.28	-
1H-Cycloprop[e]azulene	36.205	0.37	-	-
δ-kadinen	36.199	-	-	0.54
Alcohols and ketones				
13-Tetradecen-1-ol acetate	61.828	-	0.12	-
13-Octadecenal	61.839	-	1.42	-
3-Nonadecane diol	66.686	-	1.73	-

Conclusions

Propolis samples obtained from different regions varied in their in chemical constituents. This variability among regions was caused by regional differences in climate, vegetation, plant resources, soil structure, and preferences of honey bees. The chemical composition of propolis is complex and varies depending on the flora of the region and the season. Plant sources of propolis should be recorded and documented in order to standardize studies. This study is the first detailed examination of propolis from the province of Bingöl. As a result, it would contribute to future standardization studies on the propolis of Bingöl and Turkey.

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Authors' Contributions

HI, BY, TS, KK, BS, RIT, TA, ASB, AG, SC, AYS, HSY and MAK contributed to the concept, design and execution of the study. HI, BY, BS, RIT and IS were in charge of laboratory analyses. BS, TS, BY, MAK, KK, HSY and IS were responsible for supervising and writing the manuscript.

Conflict of Interest Declaration

The authors declare that there is no conflict of interests in the publication of this manuscript.

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