Apiterapi ve Doğa Dergisi
Journal of Apitherapy and Nature
www.dergipark.gov.tr/jan

Investigation of Phenolic Content of Propolis Produced in Yigilca District of Duzce Province in Western Black Sea Region of Turkey

Türkiye'nin Batı Karadeniz Bölgesinde Düzce İli Yığılca İlçesinde Üretilen Propolisin Fenolik İçeriğinin Araştırılması

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Received/Geliş Tarihi: XX/XX/20XX, **Accepted**/ Kabul Tarihi: XX/XX/20XX

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Abstract

With the increase of consumer consciousness in recent years, the use of natural products has gained importance in many sectors such as food, medicine, cosmetics and Propolis is one of these natural products. The purpose of this study was to investigate the chemical compositions of propolis samples from the Yigilca town of Duzce province of in the western Black Sea Region of Turkey due to its medical and biological availability. Raw propolis collected from plastic propolis traps put into top of the hive from different apiaries and Duzce University Beekeeping Research, Development and Application Center (DAGEM). Ethanolic propolis extract was prepared and analysis was performed using a high-performance liquid chromatography with diode-array detector (HPLC-DAD). The results demonstrated that Biochanin, Gallic Acid, CAPE (Caffeic acid phenyl ester), Pinostrobin and Pinobankstin were seen dominantly and they belong to the flavonoid group. It has been observed that all of the samples contained different bioactive compositions at different levels. It was observed that the chemical contents were different even in the samples obtained from the same region. In conclusion, the contents and properties of the propolis samples obtained from the same region may be different although the chemical content of propolis is largely dependent on many factors such as flora, geographical location, honeybee subspecies, solvent. So it is important to understand chemical compound for the propolis standardization in beekeeping.

Keywords: Bioactive Compounds of Propolis, HPLC-DAD

Özet

Son yıllarda tüketici bilincinin artmasıyla birlikte, gıda, ilaç, kozmetik gibi pek çok sektörde doğal ürünlerin kullanımı önem kazanmıştır ve Propolis bu doğal ürünlerden biridir. Bu çalışmanın amacı, tıbbi ve biyolojik yararlığı nedeniyle, Türkiye'nin batı Karadeniz Bölgesinde yer alan Düzce İli Yığılca ilçesinde üretilen propolis örneklerinin kimyasal bileşimlerini araştırmaktır. Düzce Üniversitesi Arıcılık Araştırma, Geliştirme ve Uygulama Merkezi'ndeki (DAGEM) ve farklı arılıklardaki kovanlara yerleştirilen plastik propolis tuzaklarından ham propolisler toplandı. Etanolik propolis ektraktı hazırlandı ve diyot dizisi detektörü (HPLC-DAD) ile yüksek performanslı sıvı kromatografisi kullanılarak analiz edildi. Sonuçlar göstermiştir ki; Biochanin, Gallik Asit, CAPE, Pinostrobin ve Pinobankstin dominant olarak bulunmaktadır ve bu bileşenler flavonoid grubuna aittir. Tüm örneklerin farklı seviyelerde farklı biyoaktif bileşimler içerdiği gözlenmiştir. Aynı bölgeden alınan örneklerde bile kimyasal içeriğin farklı olduğu gözlenmiştir. Sonuç olarak, propolisin kimyasal içeriği flora, coğrafi konum, bal arısı alt türleri, çözücü gibi birçok faktöre bağlı olmasına rağmen, aynı bölgeden elde edilen propolis örneklerinin de içeriği ve özellikleri farklı olabilir. Bu nedenle arıcılıkta propolis standardizasyonu için kimyasal bileşiğin anlaşılması önemlidir.

Anahtar kelimeler: Propolisin Biyoaktif Bileşenleri, HPLC-DAD

1. INTRODUCTION

Propolis collected from the flowers of the plants is a resinous substance, which generated enzymatically resinous substances and plant secretion by worker bees as the result of biochemical changes and it is semi-solid at room temperature and its color ranging from yellow to dark brown (Anonym, 1989). Propolis is used by bees to narrow the hive entrance, for the repair of honeycomb, to facilitate vulnerability and to cover holes and cracks in the hives. Propolis is a disinfectant substance, which allows disinfection of cells. Propolis contains more than three hundred components that include polyphenols, its esters, alcohols, steroids, amino acids and inorganic compounds, which have an extensive diversity of pharmaceutical properties and biological activities (Padmavathi et al. 2006a; Kanbur et al. 2009). So its antimicrobial, anti-inflammatory, antiviral, antitumoral, antiparasitic and antioxidant properties is known since ancient times (Moreno et al. 2000; Padmavathi et al. 2006b; Paulino et al. 2008; Sforcin 2007; Yousef et al. 2003, 2004;). It is believed that propolis has these properties due to the phenolic compounds included in it.

Phenolic compounds are molecules formed by the direct attachment of one or more hydroxyl groups to an aromatic ring and they are separated into phenolic acids and flavonoids as two main groups. Phenolic acids are classified as hydroxybenzoic acids and hydroxycinnamic acids in two; flavonoids are divided into six subgroups: catechins, anthocyanins, flavanons, flavonols, flavones and isoflavonoids. Phenolic compounds such as flavonoids exist as active ingredients in many plants, traditional medicines and natural products such as propolis and to prevent and treat against diseases (Wadsworth & Koop, 1999). Furthermore, extraction of new sources of flavonoids, acquisition of flavonoids, determination of their properties and extension of their uses are the most recent and one of the important problems. But synthetic production of flavonoids with a wide range of uses is currently not carried out, so only one source is flavonoid-containing plants and propolis. It is considered that the chemical content and properties of propolis are largely dependent on the beekeeping period, the season, the harvesting method and the type of vegetation in the geographical region (Farre et al. 2004). Therefore, it is important to understand chemical compound for the propolis standardization in beekeeping, because it can potentially contribute to the more efficient distribution of bee hives.

The content of propolis varies depending on the content of the plants in the area where it is collected. Therefore, the chemical structure and pharmacological activities of propolis samples belonging to different geographical regions of the world need to be examined and determined. The geographical difference of the chemical composition of propolis revealed the necessity of investigating the propolis belonging to region. Examples from different geographical regions include different chemical compositions. The main difficulty encountered is that there is no control at the origin of the propolis. This variability causes significant problems in the quality control and medical use of propolis. New research studies are needed to determine the chemical structure of propolis and the biological activity of the substances isolated from it. Especially in different geographical regions it is important to discover propolis plant sources. This approach requires the investigation of the biological activity and chemistry of propolis in a wide area.

Since Yığılca propolis is considered to be evaluated in the pharmaceutical sector, we aimed to reveal the content analysis, biological and chemical properties. There are many investigations about the chemical compositions of propolis samples from different provinces of Turkey (Table 1). But no report is found related to the chemical composition of Duzce province in Western Black Sea Region of Turkey. Therefore, considering the importance of natural products for their remedial and therapeutic value, in the present study was aimed to investigate the chemical compositions of Duzce with rich vegetation.

Table 1. Investigations about the chemical composition of propolis samples from different provinces of Turkey

Reference	Province	Compounds*
Celemli (2015)	Kirklareli, Tekirdag, Istanbul, Zonguldak, Bursa, Canakkale, Kastomonu, Ankara, Antalya, Hatay, Kayseri, Malatya, Elazig, Tunceli, Sivas, Erzincan, Trabzon, Rize, Artvin, Ardahan	Ethyl oleate, Hexadecanoic acid ethyl ester, 4H-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-phenyl, Chrysin, 2-propen-1-one,1-(2,6-dihydroxy-4-methoxyphenyl)3-phenyl, Pinocembrin, 17-pentatriacontene, 1-Nonadecene, Tricosane, Z-14-Nanacosane, Z-12-Pentacosane, 1-Hexacosane, Nonadecane, Octadecane, 2-Nonadecanone, Carboxylic acids, Benzoic acid, 2-methoxy-4-vinylphenol, Phenylethyl alcohol, Benzenemethanol, 2-buten-1-ol,2-methyl, 3-buten-1-ol,3-methyl, 2-Naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-,(2R-(2.alpha.,4a..alpha.,8a.beta.)
Aliyazicioglu R et al. (2013)	Kastamonu, Eskisehir, Eregli, Zonguldak, Izmir, Erzurum, Sinop, Artvin	Gallic acid, Proto-catechuic acid, p-OH benzoic acid, Chlorogenic acid, Vanillic acid, Syringic acid, Caffeic acid, p-Coumaric acid, Ferulic acid, Benzoic acid, o-Coumaric acid, Abscisic acid, t-Cinnamic acid, Quercetin, Epicatechin, Rutin
Celemli et al. (2012)	Tekirdag	Major compounds: Pinostrobin, chalcone, Tetrochrysin, Pinocembrin, Galangin, Chrysin, Acacetin, Heptadecanoic acid,15-mehyl-ethyl ester, 4-Pentenoic acid, 5-phenyl, Benzoic acid, 4-pentenoic acid, 5-phenyl-cyclopropancarboxylic acid, 2-phenyl-, methyl ester, 2-butenoic acid, 2-methyl, pentadecanoic acid ethyl ester, 1,2-benzenedicarboxylic acid diis ooctyl ester. Hydrocarbons group, cinnamic acid and its esters group were observed in minor ratios.
Duran et al. (2011)	Bursa, Hatay	3-Methyl-3-buten-1-ol, Phenylethyl alcohol, (E)-11-Hexadecen-1-ol, 2-Propen-1-ol, 2-Naphthalene-methanol, 13-Tetradecy-11-yn-1-ol, Olean-12-en-3-ol, Benzenemethanol, 5-Phenyl-4-pentenoic acid, Benzoic acid, Benzenepropanoic acid, 3-Phenyl-2-propenoic acid, Decanoic acid, 9-Octadecenoic acid, Octadecanoic acid, Benzene acetic acid, 4-hydroxy-3-methoxymethyl ester, Octadecanoic acid-methyl ester, 1,2-Benzenedicarboxylic acid, bis(8-methyl nonyl) ester, 1,2-Benzenedicarboxylic acid, bis(8-methyl propyl) ester, 1,2-Benzenedicarboxylic acid, butyl 8-methylonyl ester, Benzyl cinnamate, 1,2-Benzenedicarboxylic acid, diisodecyl ester, Benzaldehyde, Tetradecanoic acid, Heptadecanoic acid, n-Hexadecanoic acid, Tetradecanoic acid, ethyl ester, Hexadecanoic acid, ethyl ester, Heptadecanoic acid, 15-methyl-ethyl ester, 4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl, 4H-1-Benzopyran-4-one, 2,3-dihydro-5, 7-dihydroxy-2-phenyl, 4H-1-Benzopyran-4-one, 3,5,7-trihydroxy-2-phenyl, Chrysin, 5,7-Dihydroxy-6-methoxy-3(4′-methoxyphenyl), 5-Hydroxy-6,7-dimethoxy-3(4′-methoxyphenyl), Cyclotetradecane, Heptadecane, 1-Heptadecane, 1-Nonadecene, 9-Tricosene, Delta-cadinene, Bicyclo(4.4.0) dec-1-ene, 6(Z) 9(E)-Heptadecane, 2-Propen-1-one, Ethyl oleate, Cinnamyl cinnamate, 3-Hydroxy-4-methoxycinnamic acid, 2(5H)-Furanone, 5,5-diphenyl, 2-Phenyl-2-tipyl-acenapthenone, 1-(2-Vinyl phenyl)ethanone, Totarolone, Hinokione, 2-Heptadecanoate
Gulcin et al. (2010)	Erzurum	Caffeic acid, ferulic acid, syringic acid, ellagic acid, quercetin, a-tocopherol, pyrogallol, p-hydroxybenzoic acid, vanillin, p-coumaric acid, gallic acid and ascorbic acid
Sahinler et al. (2009)	Hatay	Benzyl cinnamate, Methyl cinnamate, Caffeic acid, Cinnamyl cinnamate, Cinnamoylglcine, α-Pinene, Indolin, 2-methylen, Cyercene, 1S-cis-Calamenen, , α-Copaene, β-Maaliene, α-Elemene, β-Eudesmol, α-Eudesmol, α-Bisabolol, Geranyl acetate, Calarene, Hexacosanoic acid, Octacosanoic acid, Triacontanoic acid, Butanedioic acid, Eicosanoic acid, Docosanoic acid, Tetracosanoic acid, 9,2,15-Octadecatrienoic acid, Octadecanoic acid, 9,12-Octadecanoic acid Nonacosane, Heneicosane, Triacosane, Hexacosane, Pentadecanone, 2-Nonadecanone
Gencay & Salih (2009)	(Anzer-Rize, Bartin-Sinop, Gumushane, Mamak-Ankara, Kazan-Ankara, Kemaliye-Erzincan, Mersin, Muğla, Orhangazi-Bursa, Tahtakopru-Bursa, Trabzon, Yalova	Aromatic alcohols, Aromatic acids, Aromatic acid esters, Aromatic aldehydes, Cinnamic acid and its esters, Napthalene and its derivatives, Fatty acids, Linear hydrocarbons and their acids, Flavonoids

Silici (2008)	Kayseri, Sivas, Mersin, Karabucak, Bursa	Phenolic Compounds, Organic Acids And Fatty Acids And Their Esters, Hydrocarbons, Quinones, Amines, Alcohols and Terpenes
Katircioglu & Mercan (2006)	Trabzon, Erzurum, Tekirdag	Aromatic alcohol, Aromatic acid, Alcoholic terpenes, Aromatic esters, Flavanones, Cinnamic acid, Flavonoids, Naringenin, Apigenin, Crysin
Mercan et al. (2006)	Denizli, Aydin, Konya, Tekirdag	Aromatic alcohol, Aromatic acid, Aromatic diol, Diphenyl amine, Alcoholic terpenes, Aromatic esters, 3-4-Dimethoxy-cinnamic acid, Flavanones, Cinnamic acid, Flavonoids, Naringenin, Apigenin, 5-Hidroxy-7-methoxy flavonone, Chrysin, Vitamine E, 2-Napthalene methanol, 2-Propenoic acid-phenyl, Phenyl ethyl alcohol, Hexadeconoic acid ethyl ester, Ethyl oleate, 9-Octadecenoic acid
Popova et al. (2005)	Yozgat, İzmir, Kayseri, Adana, Erzurum, Artvin	flavonoid aglycones (pinocembrin, pinobanksin, pinobanksin 3-O-acetate, chrysin, galangin) phenolic acids (p-coumaric, ferulic, caffeic) and esters (pentenyl caffeates, benzyl and phenethyl esters of caffeic, ferulic and p-coumaric acids, cinnamyl cinnamate, diterpenic acids (dihydroabietic, abietic, isopimaric, hydroxy fatty acids (hydroxypalmitic, hydroxystearic), and triterpenic alcohols, glyceryl-, monoacetylglyceryland diacetyilglyceryl- esters of p-coumaric, ferulic and caffeic acid, together with 1,3-di-p-coumarouyl-2-acetyl glycerol, 1,3-diferuloyl-2-acetyl glycerol, 1-p-coumaroyl- 3-feruloyl-2-acetyl glycerol and 1-p-coumaroyl-3-caffeoyl-2-acetyl glycerol
Uzel et al. (2005)	Bursa, Bartin, Ankara, Trabzon	Benzyl alcohol, Pheny ethanol, 2-methoxy-4-vinylphenol, 2-napthalenemethanol, 5-azulenementhanol, 1-naphtlenemethanol, Bisabolol-alpha, 2-phenanthrenol, Benzoic acid, Benzenepropanoic acid, 4-pentenoic acid, 5-phenyl, Ferulic acid, Caffeic acid, 2-propenoic Acid,3-phenyl, 2-propenoic acid, 3-(4-methoxyphenyl), 1-phenanthrenecarboxylic acid, Benzaldehyde, Cinnamyl cinnamate, Benzyl cinamate, Benzyl benzoate, 1-3-hydroxy-4-methoxycinnamic acid, Lauric acid, Myristic acid, Palmitic acid, Oleic acid, Stearic acid, Linoleic acid, Cyclohexadecane, Nonadecane, Octadecane, Octadecanoic acid, Isalpinin, Pinocembrin, Pinostropin, Naringenin, 4',5-dihydroxy-7-methoxyflavanone, Chrysin, 3,4',7-trimethoxy flavanone, Hexadecanol, Pinobanksin and its derivatives, Quercetin and its derivatives, Galangine and its derivatives, Apigenin and its derivatives
Kartal et al. (2002)	Ankara, Mugla	Butanedioic acid, Malic acid, Dodecanoic acid, Hexadecanoic acid, Benzoic acid, Cinnamic acid, Ferulic acid, Caffeic acid, Caffeic acid isomer-1, Caffeic acid isomer-2, Oleic acid, α-Terpineol, 1H-3A,7-Methanoazulene, 2,3,4,7,8,8A-hexahydro, 3,6,8,8-tetramethyl-[3R-(3α,3a,7, 8aα)], 8-H-Cedran-8-ol, 4-H,5α-Eremophi1-1(10)-ene, Farnesol, 1-Naphthalenemethanol, decahydro-1,10-dimethyl-6-, methenyl-5-(5-hydroxy-3-Pentene), Thunbergol, Isopimaric acid, Dehydroabietic acid, Abietic acid, β-D-Galactofuranose, D-Fructose, α-D-Mannopyranose, Mannose, 3-α,5--Pregnan-20-one, Androstan-1,17-dimethyl-17-hydroxy-3-one, Glycerol, 1,4-Anhydroglucitol, Docosa-8,14-diyn-cis-1,22-diol, 1-(5-Ethenyltetrahydro-5-methyl-2-furanyl)-1-methylethanol
Sorkun et al. (2001)	Trabzon, Erzurum, Gumushane, Bursa	Alcohols, Aliphatic acids, Amino acids, Aromatic acid esters, Aromatic acids, Aromatic aldehyde, Flavonoids, Ketones, Others, Terpenoids, Vitamin A

2. MATERIALS AND METHODS

All propolis samples used for the present study were obtained from Yığılca town of Duzce province in western Black Sea and were kept in the freezer (-20ºC) until analysis. Ethanolic extract of propolis (EEP) was prepared as performed according to Kosalec et al. (2004) with minor modifications. In briefly, propolis samples were grounded into powders. The active components were then extracted with ethanol 96%. 10 g of each propolis sample was mixed with 100ml ethanol and left over night with continuous stirring at 300 rpm. The suspension was then filtered using qualitative filter paper. Then the raw extract or EEP was obtained. The resultant resinous product was dissolved in 96% ethanol.

The method of this study is modified version of Yang et al. (2013). For the simultaneous determination of 17 components, analysis was performed using HPLC-DAD.

All statistical analyses were performed with the SPSS 16.0 for Windows (SPSS Inc., Chicago). Results are shown as mean values and standard error.

3. RESULTS AND DISCUSSION

The chemical compositions of 70% EEP extracts from nine Yigilca propolis samples were analyzed by HPLC-DAD. Individual of 17 bioactive compounds were analyzed by high performance liquid chromatography with Diode-Array and its results were shown in Table 2. It has been observed that all of the samples contained different bioactive compositions at different levels.

P3 (3rd sample) sample was the richest in 3-4 Dimethoxycinnamic acid, Apigenin, Protocatheuic Acid, Biochanin, Caffeic acid phenyl ester, Coumaric Acid. P1 was rich in Naringenin, Trans-Cinnamic Acid, Kaempherol, Pinobankstin than other samples. Also, maximum values of Catechin, Rosemarinic acid and Gallic Acid were seen in P6 sample. Gallic acid commonly known standard antioxidant substance was found more in P6 sample (40000 µg/ml) than other samples. Biochanin, Kaempherol, Pinobankstin and Trans ferulic acid only were seen in P1, P2 and P3 samples.

Table 2. Chemical composition of 70% EEP extracts of nine Yigilca propolis by HPLC-DAD

	Descriptive Statistics				
	Compounds	**Min. (µg/ml)**	**Max. (µg/ml)**	**Mean**	
		Statistic	**Statistic**	**Statistic**	**Std. Error**
Phenolic acids	3-4Dimethoxycinnamic Acid	47.00	8440.00	1316.55	901.46
	Protocatheuic Acid	971.00	1601.00	1350.40	117.54
	TransCinnamic Acid	15.00	1149.00	398.37	163.49
	Coumaric Acid	7.00	772.00	310.66	133.28
	Ferulic acid	20.00	842.00	209.16	128.10
	Rosemarinic Acid	103.00	608.00	358.00	75.33
	TransFerulic Acid	454.00	473.00	465.33	5.78
	Gallic Acid	9.00	40000.00	**8636.88**	4710.73
Flavonoids	Biochanin	3937.00	74352.00	**28001.33**	23181.01
	CAPE	44.00	50405.00	**6122.55**	5543.08
	Catechin	283.00	4000.00	1190.14	558.57
	Hesperidin	48.00	926.00	289.60	161.13
	Kaempherol	250.00	277.00	263.50	13.50
	Pinobankstin	2701.00	3836.00	3182.33	338.78
	Pinostrobin	761.00	11903.00	**3766.11**	1265.40
	Apigenin	40.00	1400.00	351.00	185.04
	Naringenin	56.00	524.00	182.55	51.33

Min: Minimum; Max: Maximum; Std: Standard

According to mean data, it was seen that propolis samples from Yigilca district of Duzce province area with Black Sea climate were rich in Biochanin (28001.33 µg/m), Gallic Acid (8636.88 µg/m), CAPE (6122.55 µg/m), Pinostrobin (3766.11 µg/m), Pinobankstin (3182.33 µg/m), Protocatheuic Acid (1350.40 µg/m), 3-4 Dimethoxycinnamic Acid (1316.55 µg/m), Catechin (1190.14 µg/m), Trans Ferulic Acid (465.33 µg/m), Trans Cinnamic Acid (398.37 µg/m), Rosemarinic Acid (358.00 µg/m), Apigenin (351.00 µg/m), Coumaric Acid (310.66 µg/m), Hesperidin (289.60 µg/m), Kaempherol (263.50 µg/m), Ferulic acid (209.16 µg/m) and Naringenin (182.55 µg/m).

Propolis is a very versatile substance used in the pharmaceutical and cosmetic industry, apitherapy centers and food sector, because of antibacterial and antioxidant effect and different chemical composition. The use of food supplements rich in phenolic compounds plays an important role in the treatment or prevention of many diseases, particularly cancer. It is used to increase body resistance against diseases due to its immunomodulatory properties (Krell, 1996). Therapeutic effects and biological activity of the propolis is closely associated with the chemical constituents of this natural bee product. Therefore the chemical structure of Duzce propolis in EEP was primarily determined by HPLC-DAD.

The results demonstrated that Biochanin, Gallic Acid, CAPE, Pinostrobin and Pinobankstin were seen dominantly and they belong to the flavonoid group.

Biochanin was only found in the Yigilca propolis according to data on the Table 1. Due to biochanin content of these samples, they can show antitumor activity (Xiao et al. 2017), anticancer activity (Sehdev et al. 2009; Seo et al. 2011; Kole et al. 2011; Bhardwaj et al. 2014), neuroprotective activity against oxidative stress (Occhiuto et al. 2009; Liu et al. 2012), improvement in behavioral and neurochemical deficits in a model for Alzheimer's disease (Biradar et al. 2014) and protection PC12 cells against Ab-induced toxicity (Choi et al. 2010), inhibitory activity of some enzymatic systems against androgen dependent diseases, such prostate hyperplasia (Bae et al. 2012).

Another flavonoid "Gallic acid" was identified in Yığılca propolis. Aliyazicioglu et al. (2013) and Gulcin et al. (2010) found gallic acid in their propolis samples in the previous studies. There are many research about effects of gallic acid such as antioxidant activity (Khunnala et al. 2009; Naghizadeh & Mansouri, 2015; Pemminati, 2015), amelioration on traumatic nerve injury (Hajimoradi et al. 2015), antidepressant effect (Moghadas et al. 2016), cytotoxicity (Oyagbemi et al. 2016), anticancer activity (Hsu et al. 2016).

From flavonoids "Caffeic acid phenyl ester (CAPE)" was observed in a very wide range (44-50405) in Yığılca propolis samples. Our results were similar to other results (Duran et al 2011; Celemli et al. 2012, 2015) collected from different regions of Turkey. It is a compound with antioxidant, anti-inflammatory, antiviral, immunostimulatory and carcinostatic, anti-damage reperfusion, anticancer properties together with being one of the biologically active components of propolis (Borelli et al. 2002; Son & Lewis, 2002). Antioxidant Activity (Alkis et al. 2015; Khan et al. 2015; Ginis et al. 2016; Akyol et al. 2015), anti-apoptotic activity (Santos et al. 2014; Wang et al. 2016), anticancer activity (Balkhi et al. 2016), effects on inflammation (Mirzoeva & Calder, 1996; Tsai et al. 2015) and Huntington Disease (Bak et al. 2016) of CAPE has been shown previously by many researchers.

Other compounds in propolis samples from Yığılca were Pinobankstin and Pinostrobin. Pinostrobin (Celemli et al. 2012) and Pinobankstin (Popova et al. 2005; Uzel et al. 2005) were found in previous studies. It has been demonstrated that Pinobankstin has anti-microbial, anti-fungal and anti-mycotic. Furthermore, antioxidant effect of pinobanksin was showed by inhibiting lipid peroxidation of mitochondrial membrane (Santos et al. 1998). Pinostrobin has anti-bacterial effect and local anesthetic.

All these results showed that Yığılca propolis should be used for some therapeutic aims such as antimicrobial and neuroprotective due to rich Biochanin, Gallic acid, Caffeic acid phenyl ester, Pinobankstin and Pinostrobin compounds

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