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Chemical Structure and Antifungal Activity of Agean Region of Propolis in Türkiye

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A B S T R A C T

The chemical composition of propolis from Aegean Uşak (Balcıdamı and Kaşbelen), Afyon (Emirdağ and Dinar), Manisa (Salihli and Kula), Denizli (Merkez and Çivril), Muğla (Milas and Merkez), İzmir (Kemalpaşa and Menemen), Aydın (Söke and Kuşadası) and Kütahya (Hisarcık and Tavşanlı) was studied in order to determine the major compounds by using GC-MS. In this study, 8 % ethanol extract of propolis prepared by mixing 920 ml of 70 % ethanol and 80 g of propolis was used. Chemical analysis of propolis extracts indicated that the propolis samples had high concentrations of the aromatic acids, esters and other derivatives which are responsible for the antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancer properties of propolis such as benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglcine besides the most common compounds as fatty acid, terpenoids, esters, alcohols hydrocarbons and aromatic acids. Also, in this research the antifungal effects of 7 concentrations (6.25, 12.5, 25, 50 ppm) of propolis ethanol extract (PEE) against *Fusarium oxysporum* was investigated in vitro conditions. Propolis was mixed alone or in combination with potato dextrose agar (PDA) medium at various concentrations. The results indicated that the mycelial growth of the tested fungi decreased with each increase in PEE concentrations. Propolis extract collected from Muğla province showed 77.81% antifungal effect *against Fusarium oxysporum* at the highest concentration (50 ppm). The lowest antifungal effect (64.52%) against the pathogen was detected in the propolis extract collected from Denizli province.

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Introduction

Propolis is a sticky gummy resinous substance collected by worker honeybees from the young shoots and buds of the certain trees and shrubs (Sforcin, 2016; Cengiz and Genç, 2021). The plants secrete it to coat the young shoots and buds in order to protect them from the adverse effects of bad weather and from the attacks of bacteria, fungi, molds and viruses. The bees collect these substances, pack them on their hind legs, and bring them back to the hive to cover the cracks and crevices, reduce the hive entrance and coat the large insects like moths, butterfly, beetles, cicadas etc. (Krell, 1996; Kurt and Şahinler, 2003). Bees also use the propolis to cover the inside of the hive and mix it with bees wax during building combs to protect the colony and larvae from the pathogen microorganisms. Propolis has a strong antibacterial, antifungal and antiviral properties (Şahinler et al., 2003; Şahinler and Kaftanoğlu 2005; Basim et al. 2006; Pereira et al. 2008). Due to these activities thousands of adult bees and developing bees (larvae, pupae) can be protected from these pathogens (Krell, 1996). Propolis has also become popular as an alternative medicine or food for the protection of human health and the prevention of diseases (Greenaway et al., 1990; Tan-No et al., 2006).

There are more than 300 bioactive components in the structure of propolis, depending on the type of bee, geographical region, collection time and plant origin (Bankova 2005). The structure of propolis consist of 50% resin, 10% essential and aromatic oils, 30% waxes, 5% pollen and 5% other substances such as fatty acids, vitamins and minerals (Popova et al., 2007; Pasupuleti et al.,2017; AL-Ani et al., 2018; Przbylek and Karpinski, 2019). The ethanolic extract of propolis (EEP) is the most widely used preparation, and over 200 compounds have been identified (Burdock, 1998). Nowadays, many publications have been made about the chemical composition, biological activity, pharmacology and therapeutic uses of propolis (Dezmirean et al., 2020; Anjum et al., 2019; Maldonado et al., 2020; Dudoit et al., 2020).

Anatolia is an enormous geographical area, which fit snugly into each other the two most important gene center among the subtropical countries in terms of plant diversity (Özcan 2014; Bonvehi and Coll, 2000; Oruç et al., 2014; Doğan and Hayoğlı, 2012; Marcucci, 1995). There have been over 10.000 plant species endemic to Anatolia.

Fungal pathogens are the most common of all plant pathogens and are the main cause of quality losses in agricultural crops. In addition, approximately 30% of diseases in all products are caused by these pathogens and they cause billions of dollars in quality losses worldwide every year (Shuping and Eloff 2017). The Fusarium genus includes many phytopathogen species that can significantly affect many crop yields (Behera et al., 2022). Fusarium species, which are among the most important phytopathogenic fungi in the world, attack a wide variety of crops such as wheat, barley, oats, rice, corn, legumes and vegetables such as potatoes, tomatoes, cucumbers, and cause various diseases such as head blight, root and crown rot and wilt (Leslie and Summerell 2008). Fusarium wilt, caused by *Fusarium oxysporum*, is a common fungal disease of the vascular system of plants (Dita et al., 2018). In addition to crop losses, some species threaten human and animal health due to the mycotoxins they produce (Desjardins et al., 1993; Windels 2000). In most cases, it is difficult and not sufficient to control pathogens with conventional and traditional methods in the management of plant diseases. Nowadays, chemical control is accepted as the most effective method in the management of plant diseases (Er, 2021). In the last decade, resistant strains of fungal pathogens have emerged as a result of the excessive and unconscious use of pesticide in the management of fungal plant diseases. (Jogaiah et al., 2018; Joshi et al., 2019). In addition, long-term systemic fungicides against plant diseases can be easily absorbed by the soil and cause residues in food, human health and pollution of the environment (Satapute et al., 2019). Therefore, the negative effects of synthetic chemicals encouraged researchers to find suitable alternative control methods (Davari and Ezazi, 2017). With the promotion of alternative methods against many plant pathogens has received popularity the use of traditional natural products containing a large number of bioactive compounds (Er, 2021). There are many alternative control studies in which biological origin compounds such as medicinal plants, propolis and biocontrol agents inhibit the in vitro growth of plant pathogenic fungi (Davari and Ezazi, 2022). In recent years, many studies have proven the antimicrobial properties of propolis against bacterial and fungal pathogens (Curifuta et al., 2012; Pazin et al., 2019; Abo-Elyousr et al., 2021; Davari and Ezazi, 2022). Several methods such as biological control, use of aromatic oils, propolis, fungicides and other methods have been investigated to minimize crop losses caused by *Fusarium* genus and other soil-borne pathogens in cultivated crops (Erdoğan et al., 2014; Erdoğan et al., 2016; Koç et al., 2018, Er, 2021; Çakar et al., 2022; Khalil et al., 2022).

Uşak (Balcıdamı ve Kaşbelen), Afyon (Emirdağ ve Dinar), Manisa (Salihli ve Kula), Denizli (Merkez ve Çivril), Muğla (Milas ve Merkez), İzmir (Kemalpaşa ve Menemen), Aydın (Söke ve Kuşadası) ve Kütahya (Hisarcık ve Tavşanlı)) provinces are located in the Aegean Region. These provinces are diversified the typical Mediterranean ecology. There are large areas of pine forest, eucalyptus, poplar trees and many kinds of fruit orchards in the region.

Although there are several studies on the chemical composition of propolis samples collected from different regions of our country about the chemical composition of propolis is produced in the Aegean region does not have enough work. Aegean Region propolis samples collected from stationary beekeepers who gathered to determine the chemical composition of propolis and detection of antifungal activity is the original value of the research. This study, it was aimed to determine the chemical composition of propolis from Aegean Region in Türkiye, also antifungal effects of propolis against *Fusarium oxysporum* was investigated *in vitro* conditions.

Material and Method

Collection of Propolis Samples and Extraction

Propolis samples Uşak (Balcıdamı and Kaşbelen), Afyon (Emirdağ and Dumlupınar), Manisa (Salihli and Kula), Denizli (Merkez and Çivril), Muğla (Milas and Merkez), İzmir (Kemalpaşa and Menemen), Aydın (Söke and Kuşadası) and from Kütahya (Hisarcık and Tavşanlı) provinces were collected from the stationary beekeepers. A total of 16 samples of 250 grams were collected separately from two different regions of each province. They were stored in the freezer until further processing. After the samples stored in the freezer were ground, 80 g of this ground propolis was taken and mixed with 920 ml of 70% ethanol. This mixture was kept in a dark room for a week, stirred 3 times a day during this period, and filtered with filter paper at the end of the period. The finally mixture was stored at 4°C until use in the study (Krell, 1996; Şahinler et al., 2003).

GC- MS Analysis

GC-MS analysis method was used to determine the chemical structure of propolis. One mg propolis extract 1% trimethylchlorosil valve (TMCS) beaker containing 50 µl of pyridine in trifluoroacetami bistrimethylsilyl +100 µl (BSTFA) to 100°C will be ready for inspection at 30 minutes the reaction is allowed to gas chromatography. A sample 1 µl GC-MS to be injected and analyzed. Gas Chromatography Analysis, Shimadzu QP2010 GC-MS device was used and methyl polysiloxane (30 m × 0.25mm x 0.25µm) column was used. Helium gas was used as the carrier gas at a flow rate of 10 mL/min. After waiting for 5 minutes at 100°C, it was increased to 150°C for 2 minutes and finally, it was increased to 280°C with an increase of 2°C per minute and kept here for 60 minutes. Injection was made in split mode at 250°C. The peak value determined in the analysis will be described with reference library (Krell, 1996; Bankova et al., 2000).

Fungal Pathogen

This study, the pathogen *Fusarium oxysporum* isolate obtained from the stock cultures of Uşak University Faculty of Agriculture, Department of Plant Protection Mycology laboratory was used. Potato Dextrose Agar (PDA) (39 g/1000 ml distilled water) was used as the medium in the study. The media were prepared in 250 ml Erlenmeyer flasks and sterilized in an autoclave at 121°C for 15 minutes.

Antifungal Activity of Propolis

Four different concentrations (6.25, 12.5, 25, 50 ppm) of 8% ethanol extract of propolis were used to determine the antifungal effect of propolis samples collected from 8 provinces against *Fusarium oxysporum*. The sterilized PDA medium was transferred to sterilized petri dishes as 15 ml and each concentration of propolis was added to the medium. Then, 5 mm discs cut with a mushroom drill were taken from the 7-day-old cultures of Fusarium oxysporum developed in PDA medium and inoculated into petri dishes. Petri dishes were incubated at 22±2°C for seven days. Control petri dishes were not processed. Only the pathogen was inoculated. On the other hand, 50 ppm of 70% alcohol was added to alcohol control petri dishes. Experiments were carried out with 5 replications. After 7 days of incubation, the colony diameters of *Fusarium oxysporum* were measured with a caliper. The antifungal effect of propolis was calculated as a percentage with the following formula (Şahinler et al., 2003; Leslie and Summerell, 2008).

The inhibition of mycelial growth was calculated as following:

Percentage of mycelial growth inhibition (%) = (dc – dt)/dc × 100

dc = average diameter of mycelial growth in control (mm)

dt = average diameter of mycelial growth in treatment (mm)

Results and Discussion

Antifungal Activity of Propolis

The effects of different doses of propolis ethanol extract (PEE) (50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm) and different provinces (Uşak, Afyon, İzmir, Denizli, Aydın, Muğla, Kütahya, Manisa) of propolis, Alcohol control (50 ppm) and control practices on mycelial development of *Fusarium oxysporum* developed in Potato Dextrose Agar (PDA) medium were determined. The results are presented in Table 1. According to control and alcohol control application, mycelial growth of Fusarium oxysporum was prevented at all doses of propolis samples collected from all provinces. When the data is evaluated, it has been clearly determined that propolis prevents mycelial growth of the pathogen at increasing ppm doses. In alcohol control application, the pathogen inhibition effect of ethanol did not exceed half of the lowest propolis dose. Therefore, it is clearly seen that propolis, which dissolves well in ethanol, has an important antifungal effect. It is seen that the increasing ppm doses of the propolises of Uşak, İzmir, Denizli, Aydın provinces compared to the alcohol control application are in a statistically different group ($P<0.05$). The 6.25 ppm and 12.5 ppm doses of Afyon, Muğla and Kütahya propolis are in the same group statistically. Low doses of Manisa propolis showed the lowest antifungal effect among the provinces. At 50 ppm dose, the antifungal effect was 73.24%. Generally, there is a linear increase in antifungal effect with increasing doses of propolis. The highest antifungal effect was recorded at the highest application dose (50ppm) of Muğla (77.81%) province propolis, followed by Aydın (75.14%), Uşak (74.55%) and Manisa (73.24%), respectively (Table 1).

According to the study results of Şahinler et al. (2003), it has been determined that propolis significantly inhibits the development of *A. apis* in terms of statistical significance ($P<0.01$). At higher doses (50 ppm, 25 ppm and 12.5 ppm), the efficacy reached 94.4% and determined that the growth of the pathogen was inhibited and the fungustatic effect was lower at lower doses. Many studies have reported that propolis has high antibacterial, antiviral and antifungal properties (Kalogeropoulos et al., 2009). Several studies have been conducted in which propolis has an antifungal effect against human pathogens *Candida albicans* and dermatophyte fungi. Propolis has also shown an antifungal effect against some plant pathogens such as *Colletotrichum gloeosporioides*, *A.alternata*, *Fusarium* sp. and *Botrytis cinerea*. (Curifuta et al., 2012). El-Kafrawy (2008) reported that ethanol extract of propolis showed higher antifungal effects against fungi at higher concentrations than at lower concentrations. In in vitro conditions inhibited mycelial growth of *Fusarium solani*, *Pythium ultimum*, and *Sclerotinia sclerotiorum* by 88.9%, 86.7% and 83.3%, respectively.

In this study, it is clearly seen that increasing ppm doses of propolis ethanol extract prevent mycelial growth of the pathogen. Likewise, it has been stated that increasing doses of propolis extracts inhibit the growth of plant pathogenic fungi (Ezazi and Davari, 2018; Çakar et al., 2022).

In recent years, the antifungal effect of propolis on different phytopathogenic fungi has been confirmed in many national and worldwide studies (Dinler et al., 2017; Pazin et al., 2019; Gregolin et al., 2019; Kim et al., 2019; Özyiğit, 2020; Abo-Elyousr et al., 2021).

These literatures in which the antifungal effects of propolis against *Fusarium* spp. were determined are consistent with the results of our study (Gregolin et al., 2019; Kim et al., 2019; Er, 2021; Çakar et al., 2022; Khalil et al., 2022).

Chemical Composition Propolis in Provinces

The chemical composition of the alcohol extracts of propolis from the Uşak, Afyon, İzmir, Denizli, Aydın, Muğla, Kütahya and Manisa provinces are summarized in Table 2 to 11. There are too many compounds listed in Table 2 to 11 to comment on them all individually, but we will briefly cover the major groups of compounds present.

Hydrocarbons, Alcohols, Carbohydrates, Phenolic compounds, Terpenes, Terpenoids, Steroids, Vitamins, Alcohols, Amino acids, Sugar acids, Enzymes, Hormones, Carbohydrates, Hydrocarbons, Aromatic organic compounds, Organic compounds, Aromatic hydrocarbons, Hydrocarbons, Elements and other chemical compounds have been identified (Table 2 to 11).

Table 1. Antifungal activity of Propolis Applications by Province (%)

Treatment	Uşak	Afyon	İzmir	Denizli
	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$
Control	0	0	0	0
Alcohol control	5.47 ± 0.74^{a*}	8.89 ± 2.03^{a*}	5.98 ± 0.84^{a*}	5.59 ± 0.90^{a*}
6.25 ppm	15.44 ± 1.97^{b}	22.85 ± 3.08^{b}	13.78 ± 1.41^{b}	12.66 ± 1.82^{b}
12.5ppm	23.97 ± 3.40^{c}	30.45 ± 2.94^{b}	22.46 ± 2.94^{c}	22.04 ± 2.31^{c}
25ppm	46.18 ± 1.02^{d}	47.02 ± 2.26^{c}	41.66 ± 1.22^{d}	41.07 ± 2.27^{d}
50ppm	74.55 ± 2.36^{e}	67.73 ± 2.23^{d}	69.15 ± 0.98^{e}	64.52 ± 0.81^{e}
Treatment	Aydın	Muğla	Kütahya	Manisa
	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$	$\bar{x} + s\bar{x}$
Control	0	0	0	0
Alcohol control	2.26 ± 1.75^{a*}	5.93 ± 1.92^{a*}	5.57 ± 1.60^{a*}	4.32 ± 0.48^{a*}
6.25 ppm	12.71 ± 1.54^{b}	17.46 ± 1.37^{b}	19.94 ± 1.76^{b}	8.85 ± 0.38^{a}
12.5ppm	27.38 ± 3.24^{c}	20.92 ± 2.59^{b}	25.27 ± 4.02^{b}	16.38 ± 0.80^{b}
25ppm	40.54 ± 2.69^{d}	33.15 ± 2.94^{c}	35.53 ± 1.39^{c}	18.87 ± 0.72^{b}
50ppm	75.14 ± 1.18^{e}	77.81 ± 2.12^{d}	66.42 ± 2.25^{d}	73.24 ± 2.96^{c}

*The values are the average on five replicates. There is no statistical difference between the same letter and numbers in the same column ($P<0.05$). $\bar{x}$: mean, $s\bar{x}$: standart error

Table 2. Carboxylic Acid Ratios of Propolis Samples (%)

Carboxylic Acid	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Butanedioic acid -Malic acid	2.6	0.59	2.23	1.27	0.88	1.05	0.05	0.11
1,4-Butanedione	-	-	-	-	-	-	-	0.26
Propenoic acid	0.04	0.04	-	0.67	0.39	0.53	0.1	1.6
2-Propenoic acid	0.15	0.36	0.54	-	0.04	0.21	-	-
Benzenepropanoic acid	0.02	0.05	-	0.07	0.13	-	-	-
Undecanoic acid	1.4	-	-	-	-	-	-	-
Isopimaric acid TMS	0.52	1.48	0.96	0.14	-	2.42	1.38	2.42
Pimaric acid TMS	0.76	0.8	0.23	0.21	-	0.67	0.56	2.4
1,10- dioic acid	0.49	-	0.12	-	-	-	-	-
Chloroacetic acid	0.89	-	-	-	-	-	-	-
Pentanoic acid	0.11	-	-	-	0.23	-	-	-
Pyrottartaric acid	0.03	-	-	-	-	-	-	-
2- Hexenedioic acid	0.06	-	-	-	-	-	-	-
Abiatic acid	0.11	3.91	3.12	1.66	-	4.51	3.13	10.05
Dehydrob abiatic acid	-	-	-	-	-	-	2.28	5.99
Nonanoic acid	0.86	-	0.13	-	0.02	-	0.5	0.03
Butanoic acid-Butyric acid	0.71	0.14	0.15	0.12	0.14	1.31	0.81	0.13
Dehydroacetic acid	0.25	-	-	-	-	-	-	-
Azelaic acid	-	0.05	-	-	-	-	-	-
Suberic acid	-	0.08	-	1.03	-	-	-	-
L-Weınsaeure	-	0.11	-	-	-	-	-	-
Phenyl hexanoic acid	-	0.17	-	-	-	-	-	-
Dicarboxylic acid	-	0.09	-	-	1.13	-	0.12	-
Aepfelsaeure	-	0.82	-	-	-	-	0.16	-
Acrylic acid	-	-	-	-	0.43	-	0.3	-
2- Carbon Saeure	-	-	-	-	0.89	-	-	-
1-Phenantrenecarboxylic acid	-	-	-	-	-	0.06	-	-
Benzeneacetic acid	-	-	-	-	-	0.12	-	-
Acetic acid	-	-	-	-	-	-	-	0.04
Heptanoic acid	-	-	-	-	-	-	0.03	-
1,2- dicarboxylic acid	-	-	-	-	-	-	1.29	0.19
5- Chlorovaleric acid	-	-	-	-	-	-	-	0.35
Oxalic acid	-	-	-	-	-	-	-	0.59
Palustric acid	-	-	-	-	-	-	-	3.17
Benzoic acid	-	0.09	0.08	0.11	0.58	0.11	0.09	0.24
Benzamid	-	-	-	-	-	-	-	0.58
2,3,4- Trihydroxy Benzoic acid	0.21		3.78	3.89	6.53	5.22	6.02	

Table 3. Fatty Acids Ratios of Propolis Samples (%)

Fatty Acids	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Tetradecanoic acid	0.02	-	-	-	-	-	-	-
Palmitelaidic acid	0.02	-	-	-	-	-	-	-
Palmitoleic acid	-	-	-	-	-	-	-	0.08
Linoleic acid	1.21	-	-	-	-	-	-	-
Hecsadecanoic acid	1.02	0.5	0.62	0.53	0.89	0.54	0.71	0.61
Phenylhexanoic acid	-	-	-	-	-	0.37	-	-
Octadecanoic acid	0.51	0.08	0.12	0.59	0.2	0.3	0.09	0.03
9- octadecanoic acid	0.41	0.29	0.45	0.46	0.17	0.25	0.22	1.44
9,12, octadecanoic acid	-	0.17	1.27	0.94	0.66	0.85	0.18	0.22
Oleic acid	1.63	1.05	1.75	1.06	1.7	1.27	1.17	2.22
Eicosanoic acid	0.28	0.11	-	0.21	0.21	0.13	0.12	-
Caproic acid	0.06	-	-	-	-	-	-	-
3-Hydroxyluric acid	-	0.16	-	-	-	-	-	-
9-Hydroxydecanoic acid	-	0.41	-	-	1.23	-	-	
9-Decenoic acid	-	1.64	-	-	0.31	-	-	-
3-Hydroxycapric Acid	-	-	0.32	-	-	0.55	0.5	-
Tricosylic acid	-	-	0.42	-	-	-	-	-
Arachidic acid	-	-	0.11	-	-	-	-	-
Ethyl linoleate	-	-	0.09	-	0.11	0.04	0.07	-
Prostaglandin	-	-	0.61	1.48	-	-	-	-
11,14-Eicosadienoic acid	-	-	-	-	-	0.05	-	-
Elemol <alfa->	-	-	-	-	-	-	0.04	-

Table 4. Organic Compound Ratios of Propolis Samples (%)

Organic Compound	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
N-trimethylsilyl-	0.46	0.76	0.97	1.21	1.8	1.19	-	-
TMS-hydroxy dehydro abietate	1.64	-	-	-	-	-	-	-
Menthol TMS	0.24	0.05	-	-	-	-	-	-
1,4-Butandione	-	0.04	-	0.09	-	0.14	-	-
2,2'-bitiophene	-	1.24	-	-	-	0.33	0.54	-
Propene	-	0.03	-	-	0.09	0.06	-	-
Dicarbaldehyde	-	-	0.53	-	-	-	-	-
2-carboxaldehyde	-	-	1.37	-	-	-	-	-
5-dicarbaldehyde	-	-	-	0.36	-	-	-	-
Butane	-	-	-	0.25	-	-	-	-
1-silacyclohexane	-	-	-	0.95	-	-	-	-
1,3,5-triene	-	-	-	0.14	-	-	-	-
Acetaldehyde	-	-	-	0.14	-	-	-	-
Resorcinol	-	-	-	-	0.04	-	-	-
2'-spiro bisone	-	-	-	-	4.26	-	2.92	0.05
Benzopyranone	-	-	-	-	-	0.94	-	-
1,4-Methanoazulene	-	-	-	-	-	-	0.37	-
2-oxabicyclo	-	-	-	-	-	-	0.19	-
Oxepin	-	-	-	-	-	-	0.55	-
2-naphthalenol	-	-	-	-	-	-	2.15	-
Cyclohexanol	-	-	-	-	-	-	0.62	-
Cyclododeca	-	-	-	-	-	-	-	1.22
5-Hydroxymethylfurfural	-	-	-	-	-	-	-	1.12
Acetamide	-	-	-	-	-	-	-	0.14
1,5-cyclooctadiene	-	-	-	-	-	-	-	0.04
Tau.Cadınol	-	-	-	-	-	-	-	0.11
Propanediamide	-	-	-	-	-	-	-	0.04
Piperazine	-	-	-	-	-	-	-	0.11
3-pyridinol	-	-	-	-	-	-	-	0.66
Methyl abietate	-	-	-	-	-	-	-	1.73
3,7-Dioxa	1.12	0.49	1.87	0.69	-	-	-	-

Table 5. Hydrocarbon Ratios of Propolis Samples (%)

Hydrocarbon	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Adamantane	2.24	1.24		4.88		2.98		
Tetra methyl tricyclo	0.25							
Propane	1.93	0.34		0.8	1.69	0.04		
1 H-inden		0.13	0.11	0.23		0.13		
Aroma dendreni		0.03						
Silas clopentane		0.16				0.54		0.06
Undecan		0.37		0.99		1.62		
Octadecan			0.32			0.37		
1 H-cyclopropa				0.38				
Germakrene B					0.06			
5a-Epoxynaphthoxepin						0.83	0.08	
2-cyclohexene								0.06
Alpha.Copaeneol								0.15
Pregnen				1.36	0.34		0.14	
D-erythrose							0.08	
Sakuranin								0.25

Table 6. Alcohols Ratios of Propolis Samples (%)

Alcohols	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Xylitol 5TMS	0.07	0.15	0.12	-	-	-	0.03	-
inositol	0.12	0.65	0.35	0.24	0.05	0.26	0.39	-
2,5-Dihydroxybenzyl alcohol	0.81	-	-	-	-	1.74	0.51	-
Methanol	1.28	2.09	3.94	2.18	2.1	1.48	0.07	-
Ethanol	3.14	2.74	3.21	2.98	3.15	4.19	1.46	3.31
Pentitol	-	0.34	-	-	-	-	-	-
Glycerine	-	1.75	0.59	1.26	1.17	1.18	3.02	-
Butanol	-	0.17	0.24	-	0.35	-	0.03	0.21
3-Methyl Penthenol	-	-	-	0.41	-	0.1	-	-
2,5-Dihydroxybenzyl alcohol	-	-	1.44	-	-	-	-	-
Decanol	-	-	-	-	0.23	-	-	-
Ribitol	-	-	-	-	0.03	-	-	-
Erythritol	-	-	-	-	-	0.07	-	-
Vanilyl alcohol	-	-	-	-	-	-	0.03	-
Bisabolol	-	-	-	-	-	-	0.11	0.04
Propanol	-	-	-	-	-	-	-	9.01
Guaiol	-	-	-	-	-	-	-	0.09
2-Hexanol	-	-	-	-	-	-	-	0.06
Mirtenol	-	-	-	-	-	-	-	0.06
Trans-carveole	-	-	-	-	-	-	-	0.06
Sclerole	-	-	-	-	-	-	-	0.24

Table 7. Carbohydrates Ratios of Propolis Samples (%)

Carbohydrates	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
D-ribofuranose	0.1	0.2	0.18	-	0.07	0.06	0.29	0.11
D-fruktose	3.7	4.82	5.55	3.9	3.99	4.56	7.35	3.12
D-galaktose	0.3	0.4	2.8	1.75	1.01	1.24	2.55	1.84
P-D-Galactofuranose	0.17	0.12	0.1	-	-	0.44	0.1	-
P-D-Glactopyranoside	-	-	-	-	0.2	-	-	-
a-D-galactopyranose	-	-	-	-	-	-	1.51	-
a-D-glucopyranose	2.92	2.62	1.36	2.7	1.18	1.01	2.13	2.43
p-D-glucopyranose	-	-	-	0.6	-	0.59	-	-
arabinofuranose	0.03	-	0.42	-	0.03	-	0.08	-
D-Glucose	-	2.27	1.65	-	0.02	-	-	-
Glucofuranoside	-	-	0.06	-	-	0.6	0.88	-
L-altrose	-	0.1	-	0.14	-	-	0.31	-
D-ribose	-	-	-	0.2	-	0.13	-	-
D-glucitol	-	-	-	-	-	-	0.04	-
D-turanose	-	-	-	-	-	-	0.4	-

Table 8. Aromatic Compounds Ratios of Propolis Samples (%)

Aromatic Compounds	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Benzene	0.51	0.06		0.14		0.05		0.21
Naphthalene	1.81	7.5	8.02	5.5				0.26
Perhydro perylene								1.03
2-Phenantrenol								
Chalcone		0.95	0.4	0.75	0.54		0.68	
Benzaldehyde						0.05		
4,7-Benzofurandione	0.27				0.18			
2,4-Diamino pyrimidine	0.1							
Oxazole	3.6		0.86	1.45			3.47	1.02
2-methylanthraquinone	2.06	1.69	2.4	3.21	0.21			
1,6-Dihydroxy methyltraquinone					3.2	4.08		
1 H-Imidazole		0.03	0.61		0.18	1.01	1.15	1.1
1 H-Indole		0.03			0.27	0.04	0.02	
Furan	1.03	0.12		3.64	1.25	3.99	3.21	2.04
4-anilinequinoline	4.78	0.23	3.81		6.72			
1-isoquinoline					0.04			
1H, 3H-quinazoline		0.11		0.21	1.79		0.75	
9,10-anthrasendione								0.79
Ionone						0.1		0.08
Phenol								0.02
9H-Carbazole	0.02	0.04					0.1	
1,3,5-triazine	0.6	0.66	0.51	0.56	0.36	0.36	0.69	0.57
Lilial	2.23	0.28					0.08	
Vanillin	0.02	0.02			0.16			0.22
Thioene							0.33	
Benzaldehyde								0.06
4H-1-benzopyran	1.58	0.24	0.64	4.85	1.23	0.66	0.87	14.32
1,4-Butandione succindialdehyde	0.03	-	-	-	-	-	-	-
Ethyl-N-benzylamine	-	0.24	-	-	-	-	-	-
6,7-dihydroxycoumarin	-	-	-	1.96	3.1	-	-	-
M-Coumaric acid					1.42	0.54	0.54	

Table 9. Phenolic Compounds Ratios of Propolis Samples (%)

Phenolic Compounds	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Ferulic acid	2.06	1.16	-	2.25	3.13	-	-	-
Isoferulate	0.78	0.26	0.69	0.62	1.65	1.09	0.27	0.11
Caffeic acid	1.18	1.15	6.19	2.56	2.73	1.31	1.65	1.34
3,5,7-trisflavone	2.76	1.43	2.35	2.22	3.58	2.3	2.76	-
Quercetin	0.91	0.37	-	0.17	-	0.39	-	0.21
Acetyloxy caffeine	5.01	6.64	-	-	-	3.12	4.81	-
Ethyl caffeine	-	-	-	-	2.64	-	-	-
Butylated hydroxytoluene	-	3.11	0.45	-	0.19	0.29	0.36	-
Cinnamic acid	1.56	1.7	1.65	2.34	2.89	1.28	0.69	20.02
Cinnamil cinnamat	1.1	1.23	1.29	2.03	2.22	1.12	1.41	1.32
3,4-dimethoxy silicate acid	0.51	-	-	-	0.11	-	-	-

In the light of the data obtained, it is important to determine both phenolic compounds and aromatic compounds and other compounds at high rates. The rates of carboxylic acids, alcohols and phenolic compounds of İzmir samples were higher than the others. Fatty acids have been determined that fatty acids in Denizli samples are higher than propolis samples collected in Kütahya province samples, Aromatic compounds, Hydrocarbons and Terpenes in samples of Uşak province, Organic compounds, Carbohydrates and Terpenes in other examples. Greenaway et al. (1990) reported that propolis contains various chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpene quinones, coumarins, steroids, amino acids and inorganic compounds. More than 160 compounds have been identified in propolis samples collected from different parts of the world, and these compounds vary according to the botanical and geographical origin of propolis. Ali and Kunugi (2020) reported that the composition of propolis can be very different depending on the geographical region and botanical origin. Like honey, propolis is known to have antioxidant, antimicrobial, anti-inflammatory and even anti-carcinogenic effects (Hossain et al., 2022).

The rates of terpenes belonging to Uşak, Kütahya, Muğla, Aydın, Afyon, Manisa, İzmir and Denizli propolis were found to be 16,57%, 16,54%, 12,25%, 11,06%, 10,46%, 9,48%, 7,1% and 6,67% respectively (Table 10).

Table 10. Terpenes and Terpenoids Ratios of Propolis Samples (%)

Terpenes and Terpenoids	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Farnesol TMS	0.97	0.6	0.64	0.87	0.34	0.28	0.36	0.12
Ergostan	0.38	0.54				0.31		
Gama selinen	3.82	1.54		8.6	0.17	1.23	1.99	
Linalool® <tetrahydro->	0.28	0.21		1.15	3.44	0.68		
Cholestane		1.51	0.53		0.54			1.77
Nerolidol		1.08						
Patchoulen <beta->			0.35			6.54	4.38	
Nootkatone					0.37			
amirin						0.15		
Caryophyllene								0.26
Isoborneol								0.17
Alpha humulene								0.07
Delta Guaiene								0.07
Ferruginol								0.31
2-naphthalenol							2.15	
1-naphthalenamine			1.13	1.14		0.83	2.22	
Azulene	0.21				0.39		0.37	
2-oxabicyclo	4.51	5.01	4.02	4.78	4.23	2.23	4.9	4.33
Ursalic acid	0.15	0.57					0.2	
Dehydro-Cycloartanol	0.14							

Kordali et al. (2009) reported that terpenes have antifungal potential against plant pathogenic fungi depending on the type and structure of the molecule and their inhibitory effect changes.

It was found that the proportions of aromatic compounds of the propolis samples of Kütahya, İzmir, Manisa, Afyon, Denizli, Aydın, Uşak and Afyon provinces were 23.79%, 22.36%, 20.87%, 19.88%, 17.25%, 12.2%, 11.89%, 10.89% respectively (Table 8). These aromatic compounds are responsible for the anti-bacterial, anti-fungal, antiviral, anti-inflammatory and anti-cancer properties of propolis (Şahinler et al., 2003; Şahinler and Kaftanoğlu,2005; Silici and Kutluca, 2005). The propolis samples collected from the Hatay region had higher concentrations of caffeic acid and sesquiterpenes than the propolis collected from Albania, Mongolia, Egypt and Bulgaria (Şahinler et al., 2003). Şahinler and Kaftanoğlu (2005) reported that the proportion of aromatic compounds in Hatay region propolis was 50.40%. Şahinler and Kaftanoğlu (2005) also reported that the rates of the sesquiterpenes are 44.84%, 39.78% and 19.09% in Hatay, Adana and Mersin respectively. This difference is due to the fact that the plant origin of the propolis samples is different.

Hydrocarbons, which have not been associated with any of the reported biological activities of propolis were identified; the varieties and rates in propolis extract from Muğla, Kütahya, Aydın, İzmir, Afyon, Manisa, Uşak, Deniz are showed in Table 5. In total, 23 hydrocarbons were determined, Şahinler and Kaftanoğlu (2005) reported that Hydrocarbons in the structure of Propolis, Mersin, Adana and Hatay have determined the types and proportions of propolis in the water. Twenty-four hydrocarbons in total have been identified.

In their study, Polat and Koçan (2006) have reported that propolis generally contains a wide variety of bioactive components such as polyphenols (flavonoid aglycones, phenolic acids and esters, phenolic aldehydes, alcohols and ketones), terpenoids, steroids, amino acids and inorganic compounds (Kartal et al., 2003). Research on the chemical composition of propolis has found that propolis contains chemical compounds such as myristic acid, benzoic acid, benzyl alcohol, vanillin, cinnamic acid, pinosembrin, pinobanksin, quercetin, galangin, apigenin, krisin, caffeic acid, acacetin, camphoride and isovanilin (Dığrak et al., 1995; Uzel et al., 2005; Salomão et al.,2004; Burdock, 1998).

In this study, the propolis samples collected from all the samples showed 10.9% to 23% of the phenolic compounds (Table 9), 8.78% to 28.15% of the carboxylic acids (Table 2), 6.5% to 15.64% of the carbohydrates (Table 7), 5.42% to 13% of the alcohols (Table 6), organic compounds (Table 4) 2.61 to 8.08%, fatty acids (Table 3) 3.1 to 5.76%, and hydrocarbons 0.3 to 8.64% (Table 5). The compounds responsible for the biological activity of propolis are thought to be flavonoids, aromatic acids and esters. Similarly, Aygun (2017) reported that the chemical composition of propolis is complex due to the presence of many components, its antimicrobial effect is related to flavonoids, phenolic acids, phenolic acid esters, and terpenes.

Kara et al. (2014), reported that propolis has been shown to play a number of biological activities besides antibacterial, antifungal, antiviral and antioxidant properties. According to results of in this study; compounds responsible for antibacterial activity flavonol, flavones, phenolic acids and esters, prenylated p-coumaric acids, lab diterpenes. It has been reported that the compounds responsible for antifungal activity are Phenocarbine, Galangin, Benzoic Acid, Salicylic Acid, Vanillin, Mono and Sesquiterpenes, Antipellin C. Antiviral Characterization Polyphenols, phenyl carboxylic acids, cinnamic acid esters, caffeic acid, quercetin, luteolin, fisetin.

According to the study results; It has been determined that phenolic compounds in propolis samples collected from different 8 provinces from the Aegean regions are between 10.9% and 23% (Table 9). Kara et al. (2014) reported the antibacterial, antioxidant, anti-inflammatoryproperties of phenolic compounds in the propolis structure. In addition, Kara et al. (2014) reported that flavonoids and flavonoids have antibacterial, anti-inflammatory properties.

Table 11. Other Chemical Compounds Ratios of Propolis Samples (%)

Steroids	Afyon	Aydın	Denizli	Kütahya	Manisa	Muğla	Uşak	İzmir
Androstenol (pheromone)	1.32	1.61	2.35	2.32	3.93	2.03	3.26	1.05
Pregnenolone			2.9					0.09
Vitamins								
Vitamin B6 3TMS	0.13	0.03						
Retinal, 9-cis-	0.11					0.13		0.17
8-Nonatetraenoic acid			0.27					
Ascorbic acid				0.12	0.35		0.49	0.32
Alkaloids								
Lamellarin	0.67	0.21	0.35	0.36	0.3	0.23	0.3	0.11
Amino acids								
Glycine	1.18	0.28	0.5	0.34	1.4	0.45	0.36	0.14
Caproic acid		0.03				0.02		
Sugar acid								
D- Glucuronic acid		0.06				0.03		
Xylonic acid		0.07						0.02
Enzymes								
Eudesmol <gama->	0.07					0.16		0.27
Tetra hydrocannabinolic acid			0.32		0.24			
Hormones								
Dehydrotestosterone								0.17
Gibberelic acid		0.1						0.12
Elements								
Osmium hydride	0.28	0.33	0.31		0.49		0.24	
Other Chemical Compounds								
5-diphenylphosphinoyl	1.12							
Ethyl Phosphoric acid	0.02	0.2	0.06			0.05		
Silane	1.22	3.46		1.85	2.7	5.46	2.28	0.2
Benzocycloheptene		0.09	0.34					
Silanol		0.57	1.16	0.92	1.86	0.96	0.19	0.12
1,4-lactone		0.05						
Ledenoxide		2.13				1.91		
4-hydroxymandelic acid			0.92	2.27	3.95	1.44		
9- tetrahydrocannabinol acid			0.26					
Phosphine			0.14				0.11	
Tris borate			0.58					
Lınalool oxıde			2.23					
Ledenoxide- (I)			1.49					
Hyocolic acid			0.07					
Methanesulfonic acid				0.37				
Cannabinol acid				0.23			0.25	
Diethyl 2-malonate					0.68			
5-diphenylphosphinoyl						0.71		
1,6-Dihydroxy							2.57	
2-octanone								0.04
Phthalaldehyde acid								0.01
Sakuranin								0.02
Ttrifluoroacetate								0.03
Hinokione								0.11
7-Hydroxy-3-methoxy								0.14
Methyl cis-secopimarate								0.47
Lauryl gallate								0.01
Formamide								0.16
Deoxycholic acid								0.1

In this research, according to the obtained data, Afyon (2.76%), Aydın (1.43%), Denizli (2.35%), Kütahya (2.22%), Manisa (3.58%), Muğla), And Uşak (2.76%) propolis samples, flavonoids and flavonones were found in different proportions (Table 9). Ferulic Acid has been reported to have anti-ulcer, liver protective properties, anti-bacterial effect (gram-positive and gram-negative microorganisms), collagen effect, collagen and elastin accumulation (Kara et al., 2014). In this study, It was determined that ferulic acid was found in different proportions in Afyon (2.06%), Aydın (1.16%), Kütahya (2.25%) and Manisa (3.13%) propolis samples (Table 9).

It has been reported that isoferric acid, Anti-Staphylococcus aureus (Staphylococcaceae gram positive bacteria species, about 20 species are caused by nosocomial infection and are also found in the human skin flora as commensal) (Kara et al., 2014). It was determined that Isoferulic acid was used in all propolis samples; Afyon (0.78%), Aydın (0.26%), Denizli (0.69%), Kütahya

(0.62%), Manisa (1.65%), Muğla (1.09%), Uşak (0.27%) and İzmir (0.11%) were found to be isoferric acid in different proportions (Table 9). Cinnamic Acid has been reported to be an anti-Staphylococcus aureus effect (Kara et al., 2014). It was determined that cinnamic acid, Afyon (1.56%), Aydın (1.7%), Denizli (1.65%), Kütahya (2.34%), Manisa (2.89%), Muğla (1.28%), Uşak (0.69%) and İzmir (20.02%) were found in different proportions (Table 9).

It has been reported that caffeic acid has anti-viral, anti-bacterial effect and anti-ulcer, anti-cancer properties. Caffeic acid was determined in all province samples in the Aegean region. Afyon (1.18%), Aydın (1.15%), Denizli (6.19%), Kütahya (2.56%), Manisa (2.73%), Muğla (1.31%), Uşak (1.65%) and Izmir (1.34%) caffeic acid is one of the most important compounds of propolis (Table 9). It has been reported that caffeic acid esters have local anesthesia, anti-viral, immunomodulatory effect, anti-cancer, liver protective properties. Caffeic acid esters were determined at different rates in Afyon (5.01%), Aydın (6.64%), Muğla (3.12%) and Uşak (4.81%) propolis samples (Table 9).

The terpenes and terpenoids found in the samples of propolis collected in the Aegean region are the most important compounds found in the propolis structure (Table 9). It has been determined that terpenes and terpenoids are present in different proportions in the propolis samples collected from different provinces from the Aegean region investigated for their antifungal properties. These ratios were determined as 7.1%and 16.57%respectively. Benzoic Acid has been reported to have bacteriostatic, antiseptic, and anti-fungal effects on bacteriostatic and bactericidal effects (Kara et al., 2014). Benzoic acid was determined at different ratios in different propolis samples; Aydın (%0.09), Denizli (%0.08), Kütahya (%0.11), Manisa (%0.58), Muğla (%0.11), Uşak (%0.09), İzmir (%0.24) (Table 2). Bisabolol was reported to have anti-inflammatory properties (Kara et al., 2014) and Uşak (0.11%) and İzmir (0.04%) were found at different rates in propolis samples (Table 6).

The aromatic compounds and esters, which are the most important compounds identified in the propolis samples, have been determined at different rates in the propolis samples collected from all the provinces in the study (Table 8). Because of the anti-fungal and anti-bacterial properties of aromatic compounds, the proportions of the propolis samples were found to be important in the study. Benzofuran, which has been shown to have an anti-cancer / anti-tumor effect, has been detected at different rates in Afyon (0.27%) and Manisa (0.18%). Vanillin has anti-fungal activity (Kara et al., 2014). In the collected Propolis samples, Afyon (0.02%), Aydın (0.02%), Manisa (0.16%) and İzmir (0.22%) proved to be different rates (Table 8). Cumaric Acid and Esters have been reported to have anti-microbial and anti-mycotic, anti-ulcer, anti- cancer, liver protective and radiation-protective properties (Kara et al., 2014). Coumaric was found in Manisa (1.42%), Muğla (0.54%) and Uşak (0.54%) proved to be different rates in propolis samples (Table 8).

Conclusion

In this study, inhibition rates of mycelial growth of Fusarium oxysporum pathogen, a fungal origin disease, were determined in petri dishes subjected to control, alcohol control, PEE 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm doses in in vitro conditions. Increasing doses of Propolis Ethanol Extract (PEE) have been found to have more of an effect on pathogen development. To determine the effects of the alcohol control group on the pathogen development of the pathogen, propolisin activity was determined according to the control group the effect of the alcohol control pathogen inhibiting mycelial growth was found to be very low.

Increasing doses of PEE have been observed in propolis samples collected from all provinces indicating that the inhibitory effect of Fusarium oxysporum pathogen on mycelial growth is increased with dose increase. Muğla province samples (50 ppm PEE) were found to have the highest antifungal effect (77.81%) on pathogen development compared to the propolis samples collected from other provinces. It has been determined that the propolis collected from all the provinces contains different kinds of Hydrocarbons, Phenolic compounds, Terpenes and Terpenoids, Carboxylic acids, Carbohydrates, Fatty acids, Aromatic compounds, Alcohols, Steroids, Vitamins, Amino acids and enzymes in different proportions.

As a result, it was determined that the lowest antifungal activity was observed in the propolis samples collected from Denizli, Kütahya, Afyon province. It has also been determined that the propolis collected from Muğla province has the highest antifungal activity.

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