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Research Article

An Investigation of The Biological Activity of Monofloral Honey Produced in South-Western Anatolia

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Abstract: In this study, monofloral honeys (chaste, thyme, citrus, and heather) which were obtained from different sources from members of Beekeeping Associations in South-West Anatolia were studied for their antioxidant capacity, total phenolic amounts, total flavonoid amounts, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and urease inhibition activities.

Antioxidant capacity of honey samples was determined by β-carotene, DPPH, $ABTS^+$ and CUPRAC activity methods. In honey samples, the highest antioxidant activity was found in citrus honey with β-carotene/Linoleic acid color opening with IC_{50}: 7.99 mg/mL, and DPPH free radical removal activity with IC_{50}: 5.28 mg/mL in thyme honey. In CUPRAC activity, it was determined that the highest activity was found in heather honey with IC_{50}: 1.69 mg/mL, in terms of $ABTS^+$ removal activity IC_{50}: 2.80 mg/mL in chaste honey, and metal chelating activity IC_{50}: 1.56 mg/mL in thyme honey.

The total phenolic and flavonoids amounts of honeys ranged from 2.31 and 27.15 (µg PEs/mg) to 4.95 and 25.24 (µg QEs/mg), respectively. In addition, AChE inhibition IC_{50}: 24.25 mg/mL in thyme honey, BChE inhibition IC_{50}: 27.93 mg/mL in thyme honey, and urease inhibition IC_{50}: 34.89 mg/mL with citrus honey were determined concerning the highest activity, consecutively.

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1. INTRODUCTION

Honey is likely known as the oldest natural sweetener food (Kaygusuz *et al*., 2016). Honey is produced by honeybee (Apis mellifera) in almost all countries of the world and is also widely consumed as a food source (Silva *et al*., 2009). It contains sugary ingredients and significant antioxidant substances. It has also many antioxidant properties and these compounds are found naturally in honey. The main natural antioxidants in foods that protect the body against harmful free radicals are vitamins (vitamins A, C and E), flavonoids, carotenoids, and polyphenols. The antioxidant activity of honey generally consists of phenolic compounds, enzymes, ascorbic acid, and peptides (Nicholls & Miraglio, 2003).

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In most studies, it was determined that there was an inverse relationship between the consumption of fruits and vegetables and the formation of certain cancer and heart diseases (Prior & Cao, 2000; Vitaglione *et al*., 2005). Today, in addition to natural antioxidants, many synthetic antioxidants have been produced; however, many studies have reported negative effects of the use of these synthetic antioxidants on the health (Ito *et al*., 1986). Therefore, the use of safer natural antioxidants instead of synthetic antioxidants has become important.

The antioxidant activity and total phenolic content of honey have been found to be parallel (Nicholls & Miraglio, 2003). The most common phenolic compounds in honey are flavonoids and phenolic acids. These phenolic compounds have been shown to play an important role in cardiovascular diseases and cancer treatments as well as antibacterial, anti-inflammatory, anti-allergic, and anti-thrombotic effects (Al-Mamary *et al*., 2002; Pyrzynska & Biesaga, 2009; Zaidi *et al*., 2019).

The enzymes in honey are formed during the processing of nectar by a bee (Badiou *et al*., 2008; Serrano *et al*., 2007). Some of these enzymes originate from plant nectar, bee throat secretion or saliva liquid, diastase (α and β-amylase), invertase (α-glycosidase), glucose oxidase, catalase, and acid phosphatase. In addition, enzymes such as AChE, BChE, urease, and peroxidase are found in a smaller amount (Bertoncelj *et al*., 2007). However, especially among these enzymes, AChE and BChE are important enzymes required for the healthy functioning of the nervous system in our body. AChE is an enzyme that is free in tissues or is compounded with phospholipids and hydrolyzes acetylcholine (Badiou *et al*., 2008).

The cause of Alzheimer's disease, an important disease of our time, is not known exactly. However, the progression of Alzheimer's disease, especially protein accumulation in the brain, disruption of nerve conduction, such as damage to brain cells plays a significant role. In addition to the stimulating effect of acetylcholine in memory, the choline acetyltransferase that allows the synthesis of acetylcholine provides a marked reduction in disease. Inhibitions of acetylcholinesterase (AChE) have been reported to be the most widely used treatment option against Alzheimer's disease (Orhan *et al*., 2006; Vinutha *et al*., 2007; Deveci *et al*., 2018). Therefore, honey containing the enzyme AChE can be considered as a supplementary food.

The aim of this study is to determine the antioxidant capacity, total phenolic amounts, total flavonoid amounts, acetylcholinesterase, butyrylcholinesterase, and urease inhibition activities of monofloral honeys produced in the South-West Anatolia Region, which is a very important region in honey production in Turkey.

2. MATERIAL and METHODS

In this study, monofloral honeys (chaste, thyme, citrus, and heather) which were obtained from South-West Anatolia (Mugla, Antalya, Aydin, and Denizli provinces) were collected from members of Honey Producer Associations. Honey samples were coded and stored in dark and room conditions until the analyses were done. The codes of used honey samples in this study are given in Table 1. In our previous study, the physicochemical components of some monofloral honeys supplied from this region were determined (Karatas *et al*., 2019).

There are several methods for determining antioxidant capacities. However, although there are many methods, a standard method that reflects the useful and antioxidant capacity has not been developed yet. Therefore, researchers state that a single method is not sufficient and several different methods are required for antioxidant capacity determination (Wong *et al*., 2006). In this study, honey's antioxidant capacity was analyzed by β-carotene, DPPH, $ABTS^{+}$ and CUPRAC activity methods. In addition, total phenolic amounts, total flavonoid amounts, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and urease inhibition activities were determined.

Table 1. The codes, origin and region of honey samples.

CODE NO	ORIGIN	PROVINCE (TOWN)
HC1, HC2, HC3, HC4	Chaste	Aydin (Cine)
HK1, HK2, HK3	Chaste	Aydin (Kocarli)
HS1, HS2, HS3	Chaste	Aydin (Soke)
KD1, KD2, KD3, KD4	Thyme	Mugla (Datca)
KK1, KK2, KK3	Thyme	Denizli (Tavas)
KU1, KU2, KU3	Thyme	Mugla (Ula)
ND1, ND2, ND3	Citrus	Mugla (Dalaman)
NF1, NF2, NF3, NF4	Citrus	Antalya (Finike)
NK1, NK2, NK3, NK4, NK5	Citrus	Mugla (Koycegiz)
PC1, PC2	Heather	Aydin (Cine)
PD1, PD2, PD3	Heather	Mugla (Datca)
PK1, PK2, PK3	Heather	Aydin (Kocarli)

2.1. Determination of the Activities of Honeys

2.1.1. *Determination of total carotenoid content*

The antioxidant activities of the honeys studied were examined according to the β-carotene linoleate model system. 40 µL of the honey samples prepared (2.5, 5, 10, 20, 40% by mass) was taken and 80 µl emulsion solution was added. The absorbance of the emulsion solution in 96-well microplates was read at 470 nm. Then, the tubes were allowed to incubate for 120 minutes in total by reading the absorbance values at 45 ^{0}C for half an hour. In addition, deionized water was added instead of honey samples and 80 µL of emulsion solution was added to the control solution. The absorbance of the control solution was read immediately as soon as the emulsion solution was added and allowed to incubate at 45 ^{0}C for half an hour at 120 ^{0}C (Sökmen *et al*., 2004; Habib *et al*., 2014). A standard solution of five different concentrations was prepared from the synthetic antioxidants BHA and the natural antioxidant α-tocopherol at 4000 ppm concentration. The reduction percentage was given according to the below Eq. (1)

Anti-radical activity (%) = [(Abs control – Abs sample) / Abs control] ×100 (1)

2.1.2. *DPPH free-radical scavenging assay*

Free radical removal activities of honey samples were determined using 1,1-diphenyl-2-picrylhydrazase (DPPH). For this purpose, 2.5, 5, 10, 20 and 40% aqueous solutions were prepared by weight (w/v). A standard solution of five different concentrations was prepared from the synthetic antioxidants BHA and the natural antioxidant α-tocopherol at 4000 ppm concentration.

0.4 mM DPPH solution was subjected to dilution with ethanol by controlling the absorbance at 517 nm. Subsequently, 40 µL of different concentrations of honey samples were placed in microplates. Then, 120 µL of ethanol and 40 µl of DPPH solution were added to incubate for 30 minutes in the dark condition. The absorbances were read at 517 nm. The absorbance results of honey samples were examined against control. Free radical removal activity was used as below and % inhibition values were calculated from these absorbance values (Burits *et al*., 2001). Inhibition activity capacities of honey were calculated with the following equation (2).

Anti-radical activity (%) = [(Abs control – Abs sample) / Abs control] ×100 (2)

2.1.3. *$ABTS^+$ cation radical removal activity determination*

Cation removal activities of honey samples were determined using $ABTS^+$ (Re *et al*., 1999). $ABTS^+$ was obtained by reaction between a prepared aqueous ABTS solution with 7 mM and 2.4 mM potassium persulfate ($K_2S_2O_8$). It was kept in the dark for 12-16 hours at room temperature. A solution of 160 µL of $ABTS^+$ was added over 40 µL of the sample at different concentrations. After 10 minutes of incubation at room temperature, absorbance was measured in a 96-well microplate reader at 734 nm. A standard solution of five different concentrations (2.5, 5, 10, 20 and 40% by mass) was prepared from the synthetic antioxidants BHA and the natural antioxidant α-tocopherol at 4000 ppm concentration. The absorbances of the samples were evaluated against control. The capability of scavenging the inhibition activity was calculated using Eq. (2).

2.1.4. *Cupric reducing antioxidant capacity assay*

Copper (II) ion reduction antioxidant capacity was determined by the method used by Apak *et al* (2004). All honey samples were studied at five concentrations (2.5, 5, 10, 20 and 40% by mass). Each of the honey samples prepared as 40 µL in ethyl alcohol was placed in a 96-well microplate. Subsequently, 60 µL of ammonium acetate buffer was mixed with 50 µL of 7,49 mM neocuprin and 50 µL of 10 mM Cu^{+2} solutions. After 1 hour waiting period, the absorbance at 450 nm was measured against the antioxidant-free reference. A standard solution of five different concentrations was prepared from the synthetic antioxidants BHA and the natural antioxidant α-tocopherol at 4000 ppm concentration.

2.1.5. *Determination of total phenolic content*

Total phenolic content was determined by Folin-Ciocalteu reagent (FCR). A standard calibration graph was obtained by measuring the absorbance values of pyrocatechol at various concentrations at 760 nm. In the same way, 250 µL of the samples were taken and 0.1 mL FCR was added and left for 3 minutes. Then, 0.3 mL of Na_2CO_3 solution was added. After 2 hours of incubation, the amounts of phenolic compounds were determined by reading the absorbances. The amounts of phenolic compounds in honey samples were expressed as µg equivalent to pyrocatechase (Öztürk *et al*., 2007; Everette *et al*., 2010; Nayik & Nanda, 2016).

2.1.6. *Determination of total flavonoid content*

Total flavonoid amounts were made according to the method by Moreno *et al*. (2000). Different concentrations of quercetin solutions were prepared and treated with aluminum nitrate and potassium acetate. Then, the standard graph was obtained by measuring the absorbance values at 415 nm.

500 µL of the analysis samples were taken and completed to 4.8 mL with methanol. Then, 100 µL of potassium acetate solution was added and left for 1 min. Finally, 100 mL of $Al(NO_3)_3.9H_2O$ solution was added. After 40 minutes of incubation, the absorbance in 415 nm was read by adding the total amount of flavonoid in honey samples equivalent to µg quercetin (Öztürk *et al*., 2007; Escuredo *et al*., 2013).

2.2. Determination of the Enzyme Activities of Honeys

2.2.1. *Anti-cholinesterase assays*

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities were measured by a spectrophotometric method according to Ellman's method (Deveci *et al*., 2018; Ellman *et al*., 1961; Boily *et al*., 2013). AChE and BChE, acetylcholine iodide (AcI) as a substrate, butyrylcholine iodide (BcI) and yellow 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) were used for the measurement of activities. For this, five different concentrations of honey solutions (2.5, 5, 10, 20 and 40% by mass) were prepared and the IC_{50} values were calculated. In this study, galantamine solution was used as the standard inhibitor.

Measurement of AChE and BChE inhibition activities was 10 µL of different concentrations of honey samples added to one well of 96-well microplate. 160 µL of 0.1M phosphate buffer and 10 µL of AChE or BChE enzyme solutions were added. Pure water was used as a control and incubated at 25 ^{0}C for 15 minutes. Then, 10 µL of DTNB solution and 10 µL of AcI were added, and kinetic absorbances were measured for 10 minutes at 412 nm (Ellman *et al*., 1961).

2.2.2. *Urease inhibition activity measurement*

Honey samples of different concentrations (2.5, 5, 10, 20 and 40% by mass) were taken 10 µL of 96-well microplates. After interacting them with 25 µL of urease enzyme, NaH_2PO_4 prepared at pH: 8.2 was added to these concentrations. 50 µL of urea solution was added as a substrate and left for incubation for 15 minutes. Then, 70 µL of sodium hydroxide and phenol reagent containing sodium nitroprusside was added to 45 µL of ammonia solution. Finally, 70 µL of sodium hypochlorous was added and the absorbance at 630 nm was measured after 50 minutes (Khan *et al*., 2004).

3. RESULTS / FINDINGS

3.1. Antioxidant Activity Results of Honey

Antioxidant activity measurements of honey samples were made by using β-carotene, DPPH, $ABTS^+$ and CUPRAC activity methods. Accordingly, the results are determined in IC_{50} values and the results are given collectively in Table 2.

3.1.1. β-*Carotene activity results*

The results are given by determining the IC_{50} values. IC_{50} values of BHA and α-tocopherol were used as a standard for the comparison of the antioxidant activity of honey samples. When these results are examined, it is seen that the antioxidant activities of the honey samples change according to their origin, the region where they grow, and the type of honey (Habib *et al*., 2014).

Considering the IC_{50} values of β-Carotene color exploration activity results, it was determined that all honey showed the highest inhibition at a concentration of 40%. According to the β-carotene discoloration activity method, the highest IC_{50} value was observed in citrus honeys. After the citrus honeys (IC_{50}:7.996), it was determined that they had chaste (IC_{50}: 9.428), thyme (IC_{50}: 14.733), and heather honey (IC_{50}: 15.667), consecutively (Table 2).

3.1.2. *DPPH free radical removal activity results*

When IC_{50} values of DPPH free radical removal activity results were calculated, it was determined that all honeys studied showed the highest inhibition at a concentration of 40%. It was seen that the highest IC_{50} value was in thyme honey. After thyme honey, it was determined to be in heather, citrus and chaste honey, consecutively. The results of the DPPH free radical removal activity of honey samples are consistent with the available literature (Meda *et al*., 2005; Ertürk *et al*., 2014; Philip & Mohd Fadzelly, 2015).

3.1.3. *$ABTS^+$ cation radical removal activity results*

In the $ABTS^+$ cation radical removal activity results, IC_{50} values of BHA and α-tocopherol were used as a standard for comparison in determining the antioxidant activities of honey samples. It was determined that all honey showed the highest inhibition at a concentration of 40%. When the IC_{50} values were calculated, it was seen that the highest inhibition value was found in heather honeys. After the heather honeys, it was determined that they had thyme, chaste and citrus honey, respectively (Table 2).

3.1.4. *Results of CUPRAC*

$A_{0.5}$ values of BHA and α-tocopherol were used as the standard for comparison in determining the antioxidant activity of honey samples. $A_{0.5}$ values were calculated according to the method given in the Cupric Reducing Antioxidant Capacity assay. When the CUPRAC results of all

honey samples were examined, it was seen that the highest absorbance value was in the heather honey, followed by thyme, chaste and citrus honeys, respectively (Table 2) (Ulusoy *et al*., 2010).

Table 2. Antioxidant Activity Results of Monofloral Honeys.

Code	β-Carotene	DPPH	$ABTS^+$	CUPRAC
	IC_{50} (mg/mL)			$A_{0.5}$ (mg/mL)
HC1	29.580±1.34	>40.00±1.44	3.690±1.12	6.002±1.22
HC2	>40.00±1.67	>40.00±2.01	3.780±1.22	4.819±1.55
HC3	26.852±1.78	-	21.063±1.56	10.760±1.28
HC4	17.908±1.34	11.570±1.92	18.952±1.53	9.565±1.56
HK1	12.985±1.24	>40.00±1.56	4.486±1.72	1.172±1.24
HK2	38.305±1.39	11.123±1.45	2.803±1.81	9.847±1.78
HK3	18.715±1.18	>40.00±1.10	16.841±1.92	9.654±1.72
HS1	30.433±1.47	>40.00±1.22	37.469±1.44	9.847±1.68
HS2	34.268±1.83	>40.00±1.24	>40.00±1.51	22.647±1.36
HS3	9.428±1.80	>40.00±1.88	26.023±1.73	9.519±1.89
KD1	>40.00±1.14	34.335±1.12	17.762±1.67	8.875±1.19
KD2	21.062±1.89	6.122±1.78	-	3.682±1.44
KD3	>40.00±1.56	>40.00±1.67	9.576±1.35	9.406±1.77
KD4	27.971±1.65	>40.00±1.62	4.058±1.14	8.700±1.83
KK1	28.763±1.78	>40.00±1.66	16.276±1.83	8.384±2.02
KK2	14.733±1.99	6.603±1.44	5.246±1.82	3.700±1.92
KK3	20.541±2.12	>40.00±1.21	6.647±2.02	8.561±1.82
KU1	>40.00±2.21	6.460±1.50	-	7.142±1.43
KU2	25.189±1.45	5.286±1.53	3.439±2.08	6.752±1.68
KU3	26.520±1.56	10.320±1.78	4.098±1.46	5.733±1.44
ND1	17.641±1.23	>40.00±1.35	5.694±1.13	12.657±1.68
ND2	12.244±1.55	22.210±1.67	12.434±1.18	>40.00±1.15
ND3	18.542±1.78	>40.00±1.14	>40.00±1.68	>40.00±1.56
NF1	7.996±1.22	>40.00±1.43	28.949±1.66	14.993±1.25
NF2	11.658±1.78	>40.00±1.53	38.768±1.12	10.568±1.19
NF3	13.066±1.20	>40.00±1.74	36.024±1.55	20.868±1.57
NF4	22.837±1.20	13.893±1.23	-	8.802±1.77
NK1	19.803±1.23	8.037±1.57	9.419±1.89	20.056±1.42
NK2	>40.00±1.88	18.635±1.34	8.685±1.34	18.372±1.49
NK3	29.045±1.56	15.218±1.87	20.445±1.13	8.633±1.77
NK4	>40.00±1.78	15.501±1.67	20.802±1.75	30.638±1.47
NK5	26.600±1.26	21.684±1.06	25.659±1.13	19.545±1.18
PC1	28.860±1.67	>40.00±1.02	5.291±1.18	4.797±1.55
PC2	18.846±1.45	>40.00±1.10	5.084±1.98	6.948±1.77
PD1	>40.00±1.98	14.631±1.32	5.319±1.44	4.452±1.54
PD2	15.667±1.12	18.208±1.41	3.189±1.77	4.893±1.78
PD3	18.303±1.57	9.846±1.52	2.812±1.17	6.583±1.27
PK1	24.869±1.54	20.548±1.87	7.347±1.82	1.694±1.63
PK2	28.266±1.08	20.436±1.56	2.827±1.41	2.602±1.13
PK3	25.447±1.64	11.094±1.34	3.452±1.86	5.121±1.88
Standards (mg / mL)				
BHA	0.0014 ±0.00001	0.0170 ±0.00018	0.0128 ±0.00050	0.0210 ±0.00001
α-tocopherol	0.0022 ±0.00004	0.0387 ±0.00023	0.0345 ±0.00047	0.0854 ±0.00001

3.1.5. *Total phenolic and total flavonoid measurement results*

Total phenolic and total flavonoid contents of all honey samples were determined according to the method given in the determination of total phenolic and total flavonoid content, respectively. The total phenolic and flavonoid results obtained are given as pyrocatechol (mg PEs/mg honey) and quercetin equivalent (mg QEs/mg honey) in Table 3, respectively.

Table 3. Total Phenolic and Flavonoid Substance Results.

Code	Total Phenolic Substance (µg PEs/mg honey)	Total Flavonoid Substance (µg QEs/mg honey)
HC1	14.06±1.45	13.37±1.23
HC2	17.19±1.23	14.08±1.56
HC3	6.56±1.56	5.90±1.13
HC4	10.31±1.44	20.06±1.24
HK1	4.06±1.12	9.82±1.67
HK2	11.56±1.56	21.22±1.77
HK3	7.65±1.89	10.19±1.55
HS1	5.81±2.04	5.02±1.12
HS2	2.31±1.88	5.33±1.67
HS3	3.44±1.68	7.13±1.83
KD1	24.69±1.20	16.28±1.42
KD2	21.14±1.13	15.21±1.55
KD3	7.81±1.67	10.57±1.72
KD4	3.44±1.93	12.19±1.12
KK1	5.31±1.24	18.33±1.14
KK2	23.44±1.22	18.93±1.77
KK3	19.12±1.16	13.90±1.56
KU1	21.56±1.66	14.70±1.34
KU2	12.81±1.89	23.26±1.22
KU3	27.15±1.34	21.39±1.73
ND1	10.43±1.18	7.88±1.55
ND2	10.41±1.45	7.56±1.78
ND3	10.88±1.83	7.31±1.52
NF1	10.94±1.77	5.68±1.22
NF2	12.19±1.39	4.95±1.56
NF3	12.19±1.99	5.46±1.78
NF4	14.06±1.55	15.95±1.12
NK1	13.21±1.78	6.15±1.68
NK2	10.91±1.35	6.99±1.82
NK3	10.94±1.78	7.55±1.77
NK4	11.56±1.26	6.79±1.45
NK5	13.44±1.34	7.86±1.82
PC1	12.69±1.55	15.77±1.99
PC2	10.31±1.43	12.06±1.91
PD1	12.81±1.42	17.39±1.23
PD2	13.44±1.10	15.70±1.57
PD3	21.56±1.83	20.82±1.88
PK1	23.42±1.45	10.66±1.34
PK2	11.31±1.34	15.64±1.55
PK3	17.81±1.13	16.86±1.78

The phenolic content of the honey samples varied between 2.31 and 27.15 (µg PEs/mg honey), as shown in Table 3. The highest phenolic content value was 27.15 (µg PEs/mg honey) with KU3 coded samples. When the flavonoid content of these honeys was examined, it was found between 5.02-23.26 (µg QEs/mg honey) and the highest value was found in KU2 sample.

The results of the phenolic activity and the total phenolic content of different origin honey samples are consistent with those in the related literature (Al-Mamary *et al*., 2002; Nayik & Nanda, 2016; Uzun, 2011).

3.2. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) Activity Results

AChE inhibition activity of four different monofloral honeys was compared and the highest activity was determined to be in thyme honeys. Activity fell in citrus honey, heather honey and chaste honey, respectively (Table 4). Also, it was determined that the highest activity in the thyme honey was the KU2 coded sample with a concentration of IC_{50}: 25.27 mg/mL. Accordingly, the studied samples were found to have lower activity in terms of acetylcholinesterase (AChE) compared to Galantamine, the standard inhibitor. However, considering that honey is a functional food, it can be said that it has a moderate activity against acetylcholinesterase (AChE).

Considering the results of BChE inhibition activity, samples of chaste honey showed low activity against BChE. It was determined that the highest activity in the thyme honey was the KD3 coded sample with a concentration of IC_{50}: 27.93 mg/mL (Table 4). These results show that thyme honeys have a high inhibition of BChE. In the same way, citrus honeys were in the second order. After the citrus honey, chaste honeys were in the third place and heather honeys were in the fourth order. Accordingly, thyme and citrus honeys have been shown to have a lower level of inhibition in butyrylcholinesterase (BChE) compared to the standard inhibitor (Galantamine). Such studies have been supported by similar literature (Philip & Mohd Fadzelly, 2015; Uzun, 2011; Wang *et al*., 2016).

When acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity results were compared, it was determined that the highest activity was in thyme honey. Compared to the honey samples studied, citrus honeys showed activity in the second place.

Table 4. Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE) and Urease Activity Results.

Code	Acetylcholinesterase (AChE) IC_{50} (mg/mL)	Butyrylcholinesterase (BChE) IC_{50} (mg/mL)	Urease IC_{50} (mg/mL)
HC1	>80.00±1.66	78.32±1.65	71.24±1.66
HC2	>80.00±1.13	>80.00±1.12	>80.00±1.67
HC3	78.55±1.11	>80.00±1.98	36.58±1.52
HC4	>80.00±1.78	>80.00±1.55	>80.00±1.21
HK1	>80.00±1.44	>80.00±1.66	>80.00±1.98
HK2	72.24±1.67	70.64±1.34	>80.00±1.66
HK3	>80.00±1.34	>80.00±1.78	>80.00±1.35
HS1	>80.00±1.82	>80.00±1.88	35.50±1.36
HS2	>80.00±1.22	>80.00±1.24	>80.00±1.62
HS3	>80.00±1.99	>80.00±1.99	42.81±1.18
KD1	74.16±1.01	>80.00±2.12	>80.00±1.19
KD2	25.24±1.67	75.23±1.45	>80.00±1.84
KD3	61.31±1.55	27.93±1.67	70.26±1.54
KD4	>80.00±1.38	>80.00±1.77	>80.00±1.44
KK1	32.93±1.78	71.65±1.88	>80.00±1.55

Table 4. *Continues.*

KK2	64.55±1.24	69.13±1.23	57.40±1.93
KK3	>80.00±1.22	>80.00±1.33	>80.00±1.21
KU1	>80.00±1.62	>80.00±1.44	35.96±1.46
KU2	25.27±1.77	39.09±1.98	48.25±1.83
KU3	66.26±1.12	>80.00±1.24	34.89±1.34
ND1	>80.00±1.50	>80.00±1.35	>80.00±1.34
ND2	>80.00±1.78	>80.00±1.55	>80.00±1.23
ND3	32.23±1.44	>80.00±1.67	>80.00±1.35
NF1	>80.00±1.35	>80.00±1.77	>80.00±1.92
NF2	49.17±1.46	>80.00±1.22	>80.00±1.64
NF3	>80.00±1.27	66.08±1.12	>80.00±1.73
NF4	>80.00±1.22	37.64±1.18	>80.00±1.77
NK1	>80.00±1.77	>80.00±1.87	>80.00±1.33
NK2	37.79±1.89	>80.00±1.34	>80.00±1.35
NK3	>80.00±1.99	>80.00±1.33	>80.00±1.39
NK4	>80.00±1.23	35.23±1.55	>80.00±1.77
NK5	47.05±1.67	75.62±1.35	>80.00±1.63
PC1	>80.00±1.34	>80.00±1.78	>80.00±1.24
PC2	>80.00±1.25	>80.00±1.24	52.43±1.78
PD1	46.03±1.66	>80.00±1.78	>80.00±1.24
PD2	71.91±1.44	>80.00±1.28	>80.00±1.82
PD3	>80.00±1.56	>80.00±1.99	>80.00±1.76
PK1	69.61±1.23	>80.00±2.10	73.35±1.53
PK2	56.47±1.43	74.92±1.34	>80.00±1.24
PK3	>80.00±1.55	>80.00±1.55	>80.00±1.77
Standard	Galantamin	Galantamin	Thiourea
(mg/mL)	0.00054±0.0001	0.0152±0.00008	0.0166±0.00025

3.3. Urease Activity Results

Urease enzyme inhibition was investigated in five different concentrations (2.5, 5, 10, 20 and 40% by mass). Enzyme activities at some low concentrations could not be calculated. The urease enzyme inhibition results of honey samples were observed in concentrations of 20% and 40% in all honeys.

The highest urease inhibition IC_{50} value of the honeys was determined in KU3 coded thyme honey with 34.89 mg/mL. When the IC_{50} values calculated for honey samples are taken into consideration, it is seen that thyme honey is highly inhibited urease than the other honeys. Chaste honeys are in the second place. Compared to other honeys subject to the study, citrus honeys showed the lowest level of urease inhibition.

4. DISCUSSION and CONCLUSION

Four different monofloral honeys (Chaste, Thyme, Citrus, Heather) with high production potential in the South-West Anatolian region have been the subject of this specific research. Monofloral honey's antioxidant capacities were revealed by β-carotene, DPPH, $ABTS^{+}$ and CUPRAC activity methods. Also, total phenolic amounts, total flavonoid amounts, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and urease inhibition activities were determined. In this study, the values obtained were compatible with those of similar studies.

Previous studies have found a positive relationship between antioxidants and anti-cholinesterase activity in plants and vegetables as these studies indicate a strong positive correlation between antioxidant and anticholinesterase activities (Philip & Mohd Fadzelly,

2015). In the analysis, it was determined that all honeys showed antioxidant activity and especially thyme honeys had higher activity than that of other monofloral honeys. In terms of enzyme inhibition activities, it was revealed that thyme honey showed higher activity than the other honeys did in the study. When the results of the phenolic activity and the total phenolic content of different origin honey samples are taken into consideration, it can be seen that thyme honeys have the highest average value with 16.58 (µg QEs/mg honey). As a result, it can be said that all the honeys used in the study, especially thyme and heather honey, have higher antioxidant properties than those of the others (Table 3).

It was determined that all monofloral honeys analyzed had a significant antioxidant capacity. It has also been found that honey samples have an effective activity against AChE and BChE enzymes. However, it was determined that it was less effective against urease inhibition than the other enzymes studied. Furthermore, especially thyme and heather honeys have been found to have higher antioxidant and enzyme capacity than the others have.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship contribution statement

Sukru Karatas: Investigation, Resources, Software, Formal Analysis and Writing. **Abdurrahman Aktumsek**: Methodology, Supervision and Validation. **Mehmet Emin Duru:** Original draft, Visualization.

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