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[Antioxidant and antimicrobial activity of East Black Sea Region honeys]

[Doğu Karadeniz Bölgesi ballarının antioksidan ve antimikrobiyal aktiviteleri]

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ABSTRACT

Objective: Honey possesses antioxidant and antimicrobial activities. Many chronic diseases are associated with increased oxidative stress caused by an imbalance between free-radical production and the antioxidant level. For that purpose, the total phenolic contents, antioxidant potentials and antimicrobial activities of nine honey samples obtained from East Black Sea Region was investigated.

Methods: The average phenolic contents for honey samples obtained from East Black Sea Region was determined according to Folin-Ciocalteu method. For evaluation of the antioxidant activity three different methods were used, the ferric reducing antioxidant power (FRAP) assay, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay and cupric reducing antioxidant capacity (CUPRAC) assay. The antimicrobial activity was studied by the disc diffusion method, using ten bacteria and three yeasts.

Results: The average phenolic content for these samples was determined as 0.224 mg gallic acid equivalents per g honey. According to FRAP assay, antioxidative activity of honeys was between 0.973 and 9.053 µmol FeSO4.7H2O/g. But the average CUPRAC activity was found as 7.815 mol Trolox/g honey. IC50 values were found as between 29.388 and 458.450 mg/mL at the end of DPPH radical scavenging activity assay. The samples showed moderate antimicrobial activity against many microorganisms.

Conclusion: All the analyzed East Black Sea Region honey samples demonstrated antioxidant and antimicrobial activity level can be considered effective.

Key Words: Honey, radical scavenging activity, antimicrobial activity, antioxidant activity, phenolic content, FRAP, DPPH, CUPRAC

Conflict of Interest: There is no conflict of interest in respect of this manuscript.

ÖZET

Amaç: Bal antioksidan ve antimikrobiyal aktiviteye sahiptir. Çoğu kronik hastalık serbest radikal üretimi ile antioksidan seviye arasındaki dengesizliğin sebep olduğu artan oksidatif stres ile ilişkilidir. Bu amaçla Doğu Karadeniz Bölgesinden elde edilen 9 bal örneğinin toplam fenolik içerikleri, antioksidan potansiyelleri ve antimikrobiyal aktiviteleri incelenmiştir.

Yöntemler: Doğu Karadeniz Bölgesinden elde edilen bal örneklerinin ortalama fenolik içrikleri Folin-Ciocalteu yöntemine göre belirlendi. Antioksidan aktivitenin değerlendirilmesi için üç farklı yöntem kullanılmıştır: demir indirgeyici antioksidan güç (FRAP) testi, 1,1-difenil-2-pikrilhidrazil (DPPH) radikali süpürme aktivitesi testi ve bakır indirgeyici antioksidan kapasite (CUPRAC) testi. Antimikrobiyal aktivite disk difüzyon tekniğine göre on bakteri ve üç maya kullanılarak incelendi.

Bulgular: Bu örnekler için ortalama fenolik içerik 1 g bal örneği için gallik asit eşdeğeri olarak 0.224 mg olarak belirlenmiştir. FRAP testine göre 1 g bal örnekleri için antioksidan aktivite 0.973 ve 9.053 µmol FeSO4.7H2O değerleri arasındadır. Ortalama CUPRAC aktivitesi troloks eşdeğeri olarak 1 g bal örneği için 7.815 mol olarak bulunmuştur. DPPH radikal süpürme aktivitesi testinin sonucunda IC50 değerleri 29.388 ve 458.450 mg/mL arasında bulunmuştur. Örnekler çoğu mikroorganizmaya karşı orta derecede antimikrobiyal aktivite göstermiştir.

Sonuç: Doğu Karadeniz Bölgesinden elde edilen çalışılan tüm bal örnekleri önemli sayılabilecek derecede antioksidan ve antimikrobiyal aktiviteye sahiptir.

Anahtar Kelimeler: Bal, radikal süpürme aktivitesi, antimikrobiyal aktivite, antioksidan aktivite, fenolik içerik, FRAP, DPPH, CUPRAC

Çıkar Çatışması: Yazarlar arasında çıkar çatışması yoktur.

Introduction

The demand for natural products is increasing day by day to a healthy nutrition, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception of this problem in recent years [1]. Honey is head of the list of these kinds of natural products. Honey, is a supersaturated solution of fructose and glucose and contains a wide range of minor constituents such as minerals, lipids, proteins, free amino acids, vitamins, organic acids, enzymes, and, volatile chemicals, phenolic compounds [2,3]. Honey is known to be rich in both enzymatic and non-enzymatic antioxidants [2,4,5]. Honey properties from different locations should be different because of the composition of active components in plants depends on various factors, particularly plant bio and chemotype and climatic conditions [1].

Many authors demonstrated that honey serves as a source of natural antioxidants, which are effective in reducing the risk of many chronic diseases such as heart disease, cancer, immune system decline, cataracts, different inflammatory processes etc. [6]. Furthermore, honey can prevent deteriorative oxidation reactions in foods such as enzymatic browning of fruit and vegetables [7], lipid oxidation in meat [4,8], and inhibit the growth of food borne pathogens and food spoilage organisms [9]. Beneficial roles of honey like these are partially attributed to its antibacterial and antioxidant activities [10]. Honey consumption has been reported to be effective in increasing the total plasma antioxidant and reducing capacity in humans [11]. The composition and antioxidant activity of honey depend on the floral source used to collect nectar by honeybee, seasonal and cilmatic factors, substantially. The dependence to various factors during processing, handling and storage is lower [12]. The variation in the profiles of antioxidant substances in honeys might be responsible for the widely varying abilities of honeys to protect against oxidative reactions [4].

The combination of honey contents suppress the growth of spoilage bacteria and contribute to the stability of the product without the necessity of particular storage conditions. However, the presence of a small number of bacteria species is anticipated in unpasteurized honeys, comprising their natural microflora [13]. On the other hand variable amounts of hydrogen peroxide and other nonperoxide factors as lysozyme, phenolic acids and flavonoids [13], free radical production, increased osmolarity, acidity, water activity, volatiles, organic acids, and beeswax, among others, are proposed by many researches as the contributing factors for its antimicrobial activity [14]. The antibacterial properties of honey were reviewed by Molan [15]. The antimicrobial activity of honeys has also been subject to extensive analysis. The interest is based mainly on the activity against pathogens and its use as a natural medicine [16]. Taken as a whole, these factors give honey unique properties and there has been an increasing interest in determination of the antioxidant and antimicrobial activity of honey, in the recent years [4, 17]. Therefore, researches on antibacterial and antioxidative activities of honey originating from different parts of the world should continue to identify new antibacterial and antioxidative agents and help clarify the healing mechanisms [18]. Because of Turkey has an important place among the honey producer countries, since it is suitable for apiculture in terms of the flowers, such studies have been performed by scientist from Turkey [19]. Thus, in the present study we aimed to determine the total phenolic content of nine honey samples provided from East Black Sea Region of Turkey as well as their antioxidant levels. Furthermore, the antibacterial potency of natural honeys against several bacterial and yeast strains were examined. Our study will contribute to a better understanding of the antioxidant and antimicrobial activities in Black Sea Region honeys.

Materials and Methods

Honey Samples

Nine honey samples harvested in 2007 obtained directly from local apiarists relating to floral region of East Black Sea were used for investigation in terms of antimicrobial activities and antioxidative capacities. The heterofloral honey samples were provided from the provinces in which the honey production is widespread. For this purpose, coastal and inland cities were also balanced. The honey samples were extracted with 95% ethanol at room temperature. The extracts were kept at 4 ˚C for a day, and they were filtered through a 0.45 µm membrane filter. Obtained filtrates were stored at -20 ˚C until analysis. Each honey sample was diluted with 70%ethanol [20].

Microorganisms tested and culture media

Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection). The antimicrobial activity of honey samples was studied using ten bacterial (four gram-positive: *Staphylococcus aureus* ATCC®25923, *Bacillus cereus* ATCC®10876, *Listeria monocytogenes* ATCC®7677, *Clostridium perfringens* ATCC®313124 and six gram negative: *Escherichia coli* ATCC®25922, *Klebsiella pneumoniae* ATCC®13883, *Pseudomonas aeruginosa* ATCC®27853, *Shigella sonnei* ATCC®25931*, Yersina enterocolitica* ATCC®27729*, Salmonella typhimurium* ATCC®14028) and three fungus (*Candida albicans* ATCC®10231, *Aspergillus niger* ATCC 9642, *Saccharomyces cerevisiae* ATCC®9763*)* species. Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and fungal cells, respectively. The

concentrations of bacterial suspensions were adjusted to 10^8 cells/mL, and fungal suspension to 10^7 cells/mL.

Antibacterial assay

For determination of antibacterial and antifungal activity, diffusion disk plates method was used [21]. For this purpose, first of all, bacterial strains grown in MHB medium for 24 h, at 37°C and fungal strains were also grown in SDB medium for 27 h, at 48 °C. Overnight cultures were diluted with broth and the final bacterial and fungal cell concentrations were adjusted to 10^8 and 10^7 cells/mL by measuring spectrophotometrically at A_{600} nm, respectively. 20 mL of MHA and SDA medium was poured into each 15 cm Petri dish and allowed to solidify. 50 µL of each diluted suspension was placed over agar in petri dishes and dispersed. Then sterile paper discs (Oxoid, CT09988, 6 mm diameter) were placed on agar to load 15 µL of each honey samples. Inhibition diameters were determined after incubation for 24 h at 37°C and 27 h at 48 °C for antibacterial and antifungal activities, respectively. All tests were made in triplicate.

Minimal inhibitory concentration (MIC)

The agar dilution method, described by Vanden-Berghe and Vlietinck [22] was used for the antibacterial screening with slight modifications. Instead of 96 well microtitre plates, 24 well tissue culture (Corning) plates were used. Honey samples were prepared by dissolving in 70% ethanol and physiological Tris buffer (1:4). The prepared samples were mixed with an equal volume of 3% agar solution at 45°C. Each of the extract samples were tested in concentrations of 50, 25, 12.5, and 6.25 mg/mL. From the test solutions 400 µL was transferred into each well of the tissue culture plate.After solidification, each well was inoculated with 10 µL of freshly prepared microbial suspension of 10^8 and 10^7cells/mL for bacteria and fungi, respectively and incubated for 24 h at 37°C. The microbial growth was assessed by a stereo microscope after the incubation period. All tests were made in triplicate.

Determination of total phenolics

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method [23] using gallic acid as standard. 20 µL of prepared ethanolic honey extracts (1 mg/mL) were mixed with 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water. The each solution was thoroughly mixed by vortexing and incubated for 3 min at ambient temperature. 400 µL of sodium carbonate solution (10%) was added to the each reaction mixture and further incubated for 2 h at ambient temperature. The absorbance of the mixtures of each honey sample were measured at 760 nm using a spectrophotometer. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0.015–0.5 mg/mL). The results were expressed as mg of gallic acid equivalents per gram of dry weight of honey samples.

Analysis of antioxidant activities

Ferric reducing antioxidant power (FRAP) assay

FRAP assay, developed by Benzie and Strain [24] as a direct method for measuring the total antioxidant power of biological fluids [5], was used in this study. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-*s*-triazine complex (Fe^{3+}-TPTZ) to its ferrous coloured form (Fe^{2+}-TPTZ) in the presence of antioxidants [17].

FRAP reagent was prepared daily by mixing 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution. A volume of 100 µl of the ethanolic honey sample was mixed with 3 mL of freshly prepared FRAP reagent. Then, the reaction mixture was incubated at 37 °C for 4 min. After that, the absorbance was determined at 593 nm against the blank that was prepared using distilled water and incubated for 1 h instead of 4 min. A calibration curve was used, using an aqueous solution of ferrous sulphate $FeSO_4.7H_2O$ in the range of 100–1000 µM. In order to make comparison, $FeSO_4.7H_2O$ was also tested under the same conditions as a standard antioxidant compound. The FRAP values were expressed as 1000 µM of $FeSO_4.7H_2O$ equivalent of g sample.

DPPH free radical-scavenging activity

The scavenging activity (H/e^- transferring ability) of honey samples against 2,2-diphenyl-1-picrylhydrazyl hydrate ($DPPH^{\cdot}$) radical was evaluated according to the method of Brand-Williams et al. [25] with minor modifications. In the presence of an antioxidant, the purple colour of DPPH decays, and the change of absorbance can be followed spectrophotometrically at 517 nm. A volume of 0.75 mL of 0.1 mM DPPH in methanol was mixed with the equal volume of the ethanolic sample solution (at different concentrations), shaken well, kept in dark for 50 min and the activity was measured at 517 nm using Trolox as standard and the values were expressed as SC_{50} (mg sample per mL), the concentration of the samples that causes 50% scavenging of DPPH radical.

Cupric-reducing antioxidant capacity

The procedure had developed by Apak et al. [26] was used. 1 mL of $CuCl_2$ solution (10^{-2} M), 1 mL of neocuproine ethanolic solutions (7.5×10^{-3} M) and 1 mL NH_4CH_3COO buffer solution (pH=7) were added to a test tube and mixed; (x) mL of phenolic extract followed by (1.1 – x) mL of water were added and vortexed. After incubating about 30 min, measurements were taken at 450 nm.

Statistical analysis

The statistical analyses were done with SPSS for Windows (v. 13.0) software. The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey HSD test. The results are evaluated in the confidence limit of 0.05.

Results and Discussion

Antimicrobial activities

Honey components such as polyphenolics, sugars, acids and hydrogen peroxide contribute to its antimicrobial activity [15]. The antibacterial properties of honey have been reviewed extensively during the last years in multiple studies all over the world [27].

In this study, we attempted to assess the value of honey from East Black Sea Region of Turkey as an antimicrobial therapeutic agent. For this purpose, disc diffusion plate method was used to ascertain the antimicrobial activities of the honey samples against 13 microorganisms. Mean diameters of inhibition zones per strain are shown in Table 1. Among the selected bacteria studied, honey samples mostly inhibited the growth of gram-negative species better than gram-positive. When MIC values (Table 2) were evaluated it can easily seen that, *S. aureus* and *S. typhimurium* were the most sensitive microorganisms, but *S. sonnei was* the least. Actually, in our study each microorganism tested exhibited different sensitivities to each of the honey samples. So, a generalization in the form of Gram-negative species are more resistant than Gram-positive could not be made. Observed antimicrobial activities were not directly related to the content of total phenolics (Table 3). This situation was attributed to the synergistic effect of the phenolic compounds with other bioactive individual components in honey [28]. Moreover, several authors have concluded that honey from certain plants has better antibacterial activity than that of others [15]. Also, it has shown that there can be a large variation in the activity of different samples from the same plant source.

According to another study have been done on *Rhododendron honeys* from Black Sea Region, the samples had no inhibitory effects on two yeasts; *C. albicans* and *S. cerevisiae* [28]. However, in our study, honey samples had inhibitory effects on yeasts sepecies. Our results are similar to the results of a study have been done on different kinds of 60 honey samples of various botanical origin by Voidarou et al [18].

Total phenolic content

Polyphenols are an important group of compounds affecting the appearance and the functional properties of honey. They are members of a class of natural compounds, recently considered of high scientific and therapeutic interest [17]. The concentration and type of polyphenolic substances in honey is variable and depends on the floral origin of honey [16]. The total phenolic content (TPC) (mg GAE/ g of honey) of honey samples examined in this study was found in the range of 0.058 to 0.396, which was determined using gallic acid as standard (r^2 = 0.997). Our results consistent with the results of a study on *Rhododendron* honeys from Black Sea Region. TPC of *Rhododendron* honeys ranged from 0.24 to 141.83 mg GAE/100 g of honey [28]. When compared with our findings, another similar level of phenolic content was also observed for Romanian honeydew honeys for which the phenolic content varied from 23.0 to 125.0 mg GAE/ 100 g [3]. Bertoncelj et al., [29] had been found that a positive linear correlation between the total antioxidant activity, determined by the FRAP method, and phenolic content. But according to results obtained in our study there was no a correlation like this. In respect of the results of another study, Gheldof et al. [2] had stated that antioxidant activity appeared to be a result of the combined activity of honey phenolics, peptides, organic acids, enzymes and Maillard reaction products.

Antioxidant activities

Food antioxidants protects our body against the oxidative damage induced by free radicals and reactive oxygen species generated in vivo as byproducts of metabolism or inflammatory processes [30]. In recent days, honey is often investigated in terms of antioxidant power as an eligible parameter for quality due to acceptance as a natural antioxidant [31].

Any specific official analytical approach for determination of antioxidant property of honey is not known. Therefore, different antioxidant assays should be used to evaluate the antioxidant properties of honeys [29, 32]. In present study, FRAP, DPPH and CUPRAC assays were preferred to evaluate the antioxidant activities of honey samples from East Black Sea region.

The reducing power test, in which the capacity of breaking radical chain reactions is reflected, is considered to be a good indicator of antioxidant capacity [33] and it is a simple direct test [29,16]. Table 3 includes the results of antioxidant activities according to FRAP assay (µmol $FeSO_4.7H_2O$/g honey). The FRAP values were found in the range of 0.973 to 9.053, which was determined using $FeSO_4.7H_2O$ as standard (r^2 = 0.996). According to Kishore et al. [34] the FRAP values may depend on the reducing capacity of the honey. The FRAP value (µmol Fe [II] per 100 g of honey) of Tualang honey obtained as 121. 89 [34] was consistent with the results of our study.

The DPPH method with the stable nitrogen centered organic radical DPPH is used for determination of free radical scavenging activity, usually expressed as SC_{50} [32, 35] the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. This means that the lower is the SC_{50} value of the sample, the higher is its antioxidant activity [35]. But, the higher the DPPH scavenging activity, the higher the antioxidant activity of the sample [32]. SC_{50} values obtained for samples used in this study were typed in Table 3. The average SC_{50} value

Table 1. Mean diameter (mm) of inhibition zones by honey samples as a result of antimicrobial activity according to disc diffusion method.

Honey Sample	Inhibition zones diameters (mm)											
	K.p.	E.c.	Y.e.	P.a.	S.t.	S.s.	B.c.	C.p.	L.m.	S.a.	A.n.	C.a.	S.c.
1	11.3±0.3 B	10.0±0.6 B	11.3±0.3 B	13.3±0.3 A	11.3±0.3 B	11.3±0.3 B	10.0±0.6 B	10.0±0.6 B	12.3±0.3 A	11.3±0.3 B	0.0±0.0 C	10.0±0.6 B	11.3±0.3 B
2	12.3±0.3 A	8.3±0.3 A	10.0±0.6 B	9.0±0.6 A	9.0±0.6 A	0.0±0.0 E	10.0±0.6 B	10.0±0.6 B	10.0±0.6 B	10.0±0.6 B	8.3±0.3 A	8.3±0.3 A	0.0±0.0 C
3	12.3±0.3 A	10.0±0.6 B	11.3±0.3 B	11.3±0.3 B	12.3±0.3 A	11.3±0.3 B	10.0±0.6 B	12.3±0.3 A	10.0±0.6 B	11.3±0.3 B	9.0±0.6 A	10.0±0.6 B	9.0±0.6 A
4	12.3±0.3 A	0.0±0.0 D	10.0±0.6 B	11.3±0.3 B	11.3±0.3 B	0.0±0.0 E	10.0±0.6 B	9.00±0.6 A	9.0±0.6 A	10.0±0.6 B	10.0±0.6 B	0.0±0.0 D	9.0±0.6 A
5	0.0±0.0 B	0.0±0.0 D	10.0±0.6 B	10.0±0.6 B	9.0±0.6 A	0.0±0.0 E	11.3±0.3 B	8.3±0.3 A	8.3±0.3 A	8.3±0.3 A	10.0±0.6 B	9.0±0.6 A	8.3±0.3 A
6	13.3±0.3 A	14.0±0.6 A	12.3±0.3 A	0.0±0.0 C	12.3±0.3 A	10.0±0.6 B	11.3±0.3 B	9.0±0.6 A	10.0±0.6 B	13.3±0.3 A	10.0±0.6 B	13.3±0.3 C	11.3±0.3 B
7	12.3±0.3 A	10.0±0.6 B	9.0±0.6 A	12.3±0.3 A	10.0±0.6 B	12.3±0.3 A	0.0±0.0 B	12.3±0.3 A	9.0±0.6 A	13.3±0.3 A	10.0±0.6 B	9.0±0.6 A	12.3±0.3 A
8	7.0±0.0	11.3±0.3 B	11.3±0.3 B	13.3±0.3 A	11.3±0.3 B	10.0±0.6 B	9.0±0.6 A	9.0±0.6 A	10.0±0.6 B	14.3±0.3 A	14.3±0.3 A	10.0±0.6 B	10.0±0.6 B
9	0.0±0.0 B	10.0±0.6 B	9.0±0.6 A	11.3±0.3 B	12.3±0.3 A	11.3±0.3 B	9.0±0.6 A	10.0±0.6 B	11.3±0.3 B	9.0±0.6 A	11.3±0.3 B	11.3±0.3 B	11.3±0.3 B
Ampicillin	13.3±0.3 A	15.3±0.3 A	15.3±0.3 A	27.0±0.0 A	22.0±0.0 A	NT	27.0±0.0 A	NT	25.0±0.0 A	11.3±0.3 A	NT	NT	NT
Cefazolin	11.3±0.3 A	16.0±0.6 C	15.3±0.3 A	24.0±0.0 A	23.0±0.0 A	NT	23.0±0.0 A	NT	32.0±0.0 A	NT	NT	NT	NT
Nystain	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15.3±0.3 A	15.3±0.3 A	NT
%70 Etanol	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The differences between the means in the same column followed by the same letter are not statistically significant, $P > 0.05$

K.p.: *Klebsiella pneumoniae,* E.c. : *Escherichia coli,* Y.e.: *Yersinia enterocolitica,* P.a.: *Pseudomonas aeruginosa,* S.t.: *Salmonella typhimurium*, S.s.: *Shigella sonnei,* B.c.: *Bacillus cereus.* C.p.: *Clostridium perfringens*, L.m.: *Listeria monocytogenes,* S.a.: *Staphylococcus aureus,* A.n.: *Aspergillus niger,* C.a.: *Candida albicans,* S.c.: Saccharomyces c*eravisiae,*

NT: No Test

Table 2. Minimal inhibitory concentration (MIC) (µg/mL) of ethanolic extracts of east Black Sea Region honeys against antimicrobial species

Honey samples	Minimal inhibitory concentrations (µg/mL)												
	K.p.	E.c.	Y.e.	P.a.	S.t.	S.s.	B.c.	C.p.	L.m.	S.a.	A.n.	C.a.	S.c.
1	12.5	25	12.5	12.5	12.5	12.5	25	25	12.5	12.5	50	25	12.5
2	12.5	25	25	25	25	50	25	25	25	25	25	25	50
3	12.5	25	12.5	12.5	12.5	12.5	25	12.5	25	12.5	25	25	25
4	12.5	50	25	12.5	12.5	50	25	25	25	25	25	50	25
5	50	50	25	25	25	50	12.5	25	25	25	25	25	25
6	12.5	12.5	12.5	50	12.5	25	12.5	25	25	12.5	25	12.5	12.5
7	12.5	25	25	12.5	25	12.5	50	12.5	25	12.5	25	25	12.5
8	25	12.5	12.5	12.5	12.5	25	25	25	25	12.5	12.5	25	25
9	50	25	25	12.5	12.5	12.5	25	25	12.5	25	12.5	12.5	12.5

K.p.: *Klebsiella pneumoniae,* E.c. : *Escherichia coli,* Y.e.: *Yersinia enterocolitica,* P.a.: *Pseudomonas aeruginosa,* S.t.: *Salmonella typhimurium*, S.s.: *Shigella sonnei,* B.c.: *Bacillus cereus.* C.p.: *Clostridium perfringens*, L.m.: *Listeria monocytogenes,* S.a.: *Staphylococcus aureus,* A.n.: *Aspergillus niger,* C.a.: *Candida albicans,* S.c.: Saccharomyces c*eravisiae*

Table 3. Total phenolic contents and antioxidative activities of honeys from East Black Sea Regıon according to FRAP, CUPRAC and DPPH assays

Honey Samples	Total phenolic content (mg GAE/g)	FRAP (µmol $FeSO_4.7H_2O$ /g)	CUPRAC (mol Troloks/g)	DPPH (SC_{50}) (mg/mL)
1	0.109 ± 0.011	2.333 ± 0.036	3.567± 0.018	286.531 ± 1.552
2	0.058 ± 0.006	2.536 ± 0.014	2.471 ± 0.095	180.967± 1.411
3	0.337 ± 0.021	9.053 ± 0.064	9.122 ± 0.012	29.388 ± 0.780
4	0.059 ± 0.012	0.973 ± 0.007	1.469 ± 0.090	458.450 ± 2.810
5	0.396 ± 0.010	4.007 ± 0.033	9.489 ± 0.060	57.306 ± 0.852
6	0.316 ± 0.000	4.630 ± 0.046	10.400 ± 0.019	52.097± 0.789
7	0.262 ± 0.023	3.345 ± 0.037	9.535 ± 0.027	79.962 ± 0.799
8	0.272 ± 0.036	4.510 ± 0.047	11.427 ± 0.044	55.909 ± 0.422
9	0.205 ± 0.015	6.073 ±0.072	12.859 ± 0.030	35.085 ± 0.643
Trolox				0.004 ± 0.001

for nine samples is determined as 137.3 mg/mL. The SC_{50} value of trolox as standard antioxidant was found as 0.004 mg/mL. Our results are consistent with the results obtained from another study on the honey samples from Black Sea Region [36]. Average antioxidant activity in terms of cupric reducing capacity (CUPRAC) was found as 7.815 mol Trolox/g honey. At the result of another study on *Turkish honeys from different floral sources,* the antioxidant activities had found with CUPRAC in range of between 124.8 and 532 µmol trolox/g [37].

The antioxidant activity of honey has been strongly correlated with the content of total phenolics [2-4]. It is easily seen from Table 3, phenolic compounds concentrations of some samples investigated in this study were almost the same, although the free radical scavenging activities were different. As a result of this, the correlation between DPPH free radical scavenging activity and total phenolics was found as 0.626. This contradiction was explained by Meda et al. [28] with these expressions. It is known that where similar phenolic

levels occur, these do not necessarily correspond to the same antioxidant responses. Furthermore, Folin–Ciocalteu assay results depends on the chemical structure of phenolics [39]. So, we can conclude that, the radical scavenging activity of a sample cannot be predicted on the basis of its total phenolic content [4]. It was reported that phenolic compounds are the main components responsible for the antioxidant effects of honey, however, non-phenolic antioxidants are also involved [2, 5].

In conclusion, several antioxidant assays and antimicrobial activity methods were used in order to evaluate the biological activities of honey samples from Black Sea Region of Turkey. According to the obtained results, all the analyzed honey samples demonstrated antioxidant and antimicrobial activity level can be considered effective, honeys obtained from Black Sea region can be used as food with therapeutic potential as antioxidant and antibacterial agent. At least, using of the honey as food supplement can yield a contribution to complement other polyphenol sources. Furthermore, the honey samples seemed to deserve other detailed investigation of to their individual_biologically active components, which may be attractive source of nutraceuticals and medicinal additives.

Conflict of Interest: There is no conflict of interest among the authors who contributed to the present study.

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