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SCREENING OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT TYPES OF HONEY SAMPLES OBTAINED FROM GEYIKLI (CANAKKALE) PROVINCE

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ABSTRACT. Honey is one of the bee product that have been considered both as foods and medicines and and it contains compounds that have antioxidant and antibacterial properties. Also it is well known that one of the factors affecting the quality of honey is antioxidant capacity. In this research, the antimicrobial activities of honey samples were screened against some bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S.aureus* ATCC 6538P, *S.aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella typhimurium* ATCC 51812) and two yeasts (*Candida albicans, Saccharomyces cerevisiae*) by using the agar well diffusion method in Mueller-Hinton Agar. Total antioxidant activity was analysed using TEAC method in honey samples that have different botanic origin. According to the results of this research, honey samples are especially effective on pathogenic strains like *Staphylococcus aureus* and *Salmonella typhimurium*. While the highest antioxidant capacity determined by TEAC method was obtained with 10.042 ± 0.12 (mM trolox / g extract) in oak honey type (H4), the lowest capacity is determined 1.800 ± 0.14 (mM trolox / g extract) in highland honey (H2). When it is compared to the other samples, the oak honey type has 3 or 5 times higher antioxidant capacity than the other honey types. So, honey may play an important role as natural antibacterial product.

1. INTRODUCTION

Honey is a nutritive food used widely in the food industry which provides energy to the organism as it has a high percentage of carbohydrates that are easily assimilated [1]. Honey contains compounds with antioxidant and antibacterial capacities, such as phenolic compounds and carotenoids. But flavonoids and polyphenols are the two main bioactive molecules present in honey which act as antioxidants. The presence of phenolic and other valuable compounds in honey also attribute many medicinal properties to it such as therapeutic, antioxidant, antimicrobial, anti-inflammatory, antitumoral, antimutagenic, antiviral and

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antiulcer activities [3].

The main antibacterial factor in honey is hydrogen peroxide which is produced by the glucose-oxidase action [1]. Researchers have been reported both bacteriostatic and bactericidal effects of honey and they are especially effective on pathogenic strains like *Klebsiella pneumonia*, *S. aureus*, *Salmonella typhimurium* etc. [4].

Honey is a natural antioxidant source because it reduces or neutralizes free radical formation. The composition of honey and its source reflects the biochemical properties of honey. The botanical origin of honey has a significant effect on its antioxidant activity [5].

The composition of honey varies depending on many factors such as the floral source, climate and environmental conditions [6]. Due to these medicinal properties, it is aimed to determine antimicrobial and antioxidant capacities of different types of honey samples obtained from Geyikli-Çanakkale province in this research.

2. Materials And Methods

2.1. Preparation of honey samples

Five different types of honey samples were obtained from Geyikli-Çanakkale (Table 2) one of the organic honey producer between July-August 2018 and transferred to Palynology Laboratory, Çanakkale Onsekiz Mart University, Biology Department. They were kept in sterile glass jars and stored at room temperature in the dark. They were described according to their different botanical origin as floral, multifloral, chestnut, oak and thymus honey by the bee-keeper.

2.2. Test microorganisms

Some bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S.aureus* ATCC 6538P, *S.aureus* ATCC 25923, *Listeria monocytogenes*, *Salmonella typhimurium* ATCC 51812) and two yeasts (*Candida albicans* and *Saccharomyces cerevisiae*) were used to evaluate the antimicrobial activity. Test organisms were obtained from first researcher's personal culture collection from Basic and Industrial Microbiology Research Laboratory in Çanakkale Onsekiz Mart University, Biology Department and were kept at +4ºC during the investigation.

2.3. Determination of antimicrobial activity

The antimicrobial activities of honey samples were screened in Mueller Hinton Agar (Merck) by agar well diffusion method [7-9]. All the bacterial strains were incubated for over-night at 37^0 C after their inoculation to Tyriptic Soy Broth (Merck) and yeast strain was incubated at 27^0 C in Malt Extract Broth. Microbial inoculum was set up to 0.5 Mac Farland before transferred to petri dishes containing Mueller Hinton Agar (MHA) and 100 µL inoculum was spread on MHA. Wells were made aseptically on MHA after the bacterial inoculation and wells were filled with honey samples approximately 50-60 µL. Afer the incubation period, inhibition zones formed around the wells on agar plates were measured by inhibition zone ruler (Bioanalyse) in mm and the results were analysed as qualitatively. While penicillin, streptomycine, ampicilline, azitromycin were used as reference antibacterial disks, the antifungal agents used for positive control were fluconazole, nystatin and ketoconazole.

2.4. Determination of antioxidant capacity

Total antioxidant activity were analysed using TEAC (trolox equivalent antioxidant capacity) method that based on the reduction of ABTS+ (2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) free radical by trolox in different types of honey samples. 0.0384 grams of ABTS+ radical was weighed and dissolved in some distilled water. 2 mL of a solution of 12.25 mM potassium persulfate (K2O8S2) was added to the dissolved ABTS + radical solution and this mixture was completed with distilled water in a 10 mL flask. The prepared radical solution was kept at room temperature in dark for 16 hours to form. The absorbance of the radical solution should be between 0.700 ± 0.2 at 734 nm in the spectrophotometer. This range was achieved by diluting the radical with PBS buffer. 1 mL of absorbed stock solution was taken over the different concentrations of honey added and it was read at the end of 6 minutes at 734 nm the spectrophotometer (Shimadzu, UV-1800, Japan). Different concentrations were determined for each honey and the analysis was performed in a parallel manner. The inhibition values of honey samples were calculated and inclined. Antioxidant capacities of honeys were calculated from trolox slope generated by spectrophotometer [10].

3. Results and Discussion

In this research, antibacterial activity was determined in different values against different bacteria strains in all the honey samples and the inhibition zones vary in the values 8-32 mm. but no zones were screened against the yeast strains. The results of antimicrobial activity were given in Table 1 according to the inhibition zone diameters formed around the wells.

When considering the results of antimicrobial activity of honey samples, it is clearly seen that *Salmonella typhimurium* ATCC51812 was the most sensitive bacteria to the all types of honey samples which were studied. The inhibition zone of *Listeria monocytogenes* only from oak honey (H4) and the biggest zone 32 mm obtained from floral honey (H1) against *Salmonella typhimurium* ATCC51812. It was found that the oak honey (H4) was more effective honey type, because its activity was measured against five bacterial strains.

However, the activity of all honey samples against *S. aureus* ATCC 29213, *S. aureus* 6538P strains is higher than the standard antibiotics discussed as reference but it is nearly two times higher against *Salmonella tyhimurium* ATCC 51812. But none of the honey samples were effective against *S. aureus* ATCC 25923.

Honey samples used in this study are especially effective on pathogenic strains like *S. aureus* 29213, *S. aureus* 6538P and *Salmonella typhimurium* ATCC51812. Many studies have been conducted about antimicrobial activity of honey types and other authors also recorded that gram positive bacteria were more sensitive to the honeys antibacterial activities than gram negative ones [11]. They are especially effective on pathogenic strains like *Klebsiella pneumonia, S. aureus, Salmonella typhimurium* etc. So it seems our results were parallel to literature.

The antioxidant capacity of honey and of its components is a very useful parameter to correlate phytochemical determinations [12]. Honey is a natural antioxidant source because it reduces or neutralizes free radical formation. The composition of honey and its source reflects the biochemical properties of honey. The botanical origin of honey has a significant effect on its antioxidant activity [5].

In this study; the antioxidant capacity of honey samples were determined which have different botanical origin (multifloral, chestnut, oak, thymus, floral) by TEAC method. Bueno-Costa et.al. were evaluated antioxidant, antimicrobial activity and were determined phenolic compounds and carotenoid contents in 24 honey samples from Brazil. They were compared DPPH and ABTS antioxidant activity assays and reported that ABTS assay was more convenient due to the highest correlations with the content of all phytochemical results than DPPH assay [13].

TABLE 1. Antibacterial activity of honey samples.

Microorganisms	**Inhibition zones (mm)***										
	H1	**H2**	**H3**	**H4**	**H5**	**AM10**	**P10**	**AZM15**	**S10**	**NY100**	**KTC50**
E. faecalis ATCC 29212	10	12	14	8	12	-	-	-	-	NT	NT
S.aureus ATCC 29213	14	18	22	18	14	12	14	18	16	NT	NT
S.aureus ATCC 6538P	-	16	18	12	18	26	30	14	16	NT	NT
S.aureus ATCC 25923	-	-	-	-	-	26	30	24	22	NT	NT
Listeria monocytogenes	-	-	-	14	-	-	-	-	-	NT	NT
Salmonella yphimurium ATCC 51812	32	30	30	26	14	14	19	21	22	NT	NT
C. albicans	-	-	-	-	-	NT	NT	NT	NT	16	30
Saccharomyces cerevisiae	-	-	-	-	-	NT	NT	NT	NT	-	-

AM10: Ampicillin (10µg), P10: Penicillin G (10 units), AZM15: Azitromycin (10µg), S10: Streptomycin (10µg), NS100: Nistatin (100 units), KTC50: Ketokonazol (50 µg), (-): No inhibition, NT: Not tested *: Values include 6 mm disk diameter

The highest antioxidant capacity determined by TEAC method was obtained with 10.04 ± 0.12 (mM trolox / g extract) in oak honey type (H4) while the lowest capacity was determined 1.80 ± 0.14 (mM trolox / g extract) in multifloral honey (H2). In addition, the antioxidant values of thyme (H5), floral (H2) and chestnut (H3) honeys were 3.05, 2.37 and 1.95 (mM Trolox/g honey), respectively. The antioxidant values are given in Table 2. In our study, when the antioxidant capacity of honey samples were compared to each other, it was seen that oak honey (H4) has 3 or 5 times higher antioxidant capacity than the other honey types. The antioxidant capacity of honey samples was shown in Figure 1.

TABLE 2. Localities and antioxidant values of honey samples.

Sample Number	**Locality**	**Type of Honey**	**Local Name**	**TEAC** (mM Trolox/g honey)
H1	Geyikli-Baspınar-Geyikbaba	Floral	Çiçek	2,375± 0,46
H2	Yaylacık District	Multifloral	Yayla	1,800 ± 0,14
H3	Ida Mountains	Chestnut	Kestane	1,952 ± 0,08
H4	Ida Mountains	Oak	Meşe	10,042 ± 0,12
H5	Akcakeçili village-Aktas District	Thyme	Kekik	3,054 ± 0,10

Can et al. evaluated antioxidant activity with FRAP (ferric reducing/antioxidant power) and DPPH (free radical scavenging activity) assay and determined phenolic components of 61 honey samples with RP-HPLC-UV. They reported that, oak, heather and chestnut honey contains the highest level of phenolic components and chestnut and oak honeys had the highest antioxidant activity with FRAP assay. They determined the highest levels of gallic acid in oak honey and total phenolic contents of oak, chestnut and multifloral honeys were 120.04, 98.26, 29.54 (mg GAE/100 g), respectively. Phenolic chemicals such as gallic acid, ferulic acid, routine, quercetin, apigenin have the capacity of different reducing [15]. These results were parallel to our results. Antioxidant activities of oak, chestnut and highland honeys were obtained as 10.05, 1.95 and 1.80 (mM troloks/g honey), respectively.

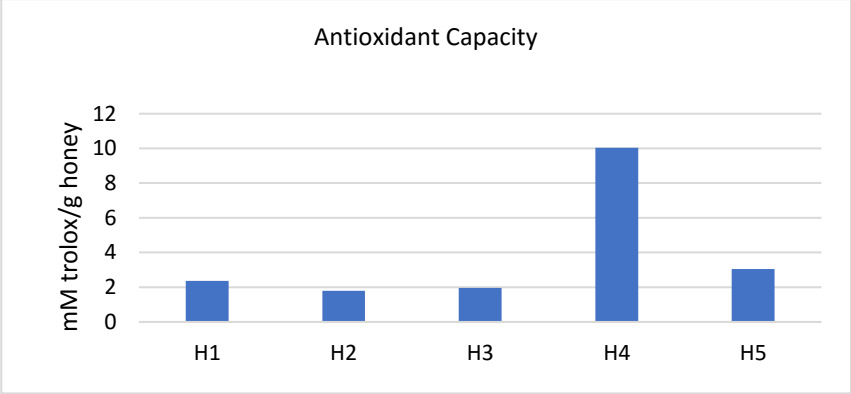

FIGURE 1. Antioxidant capacity of honey samples.

Many research reported that chestnut honey has the highest antioxidant capacity than many honey types [15-17]. But according to our results, oak, thymus and floral honeys showed higher capacity than chestnut honey. The second highest antioxidant activity in our study was determined in thyme honey. Sunflower, coriander, eucalyptus, thyme, lavender and lime honey in antioxidant activity in a study determined the highest antioxidant capacity was found in thyme honey [18]. Also the authors reported that total phenolic content exhibited a similarity with antioxidant activity. In another study, antioxidant capacity was calculated with ascorbic acid equivalent and was determined the highest antioxidant capacity in chestnut, multifloral and thyme honey, respectively [17]. In our research, the highest antioxidant activity determined in thyme, multifloral, chestnut, respectively. The results of the antioxidant capacity of three honey samples were opposite from Sağdıc et al. The reason of these results may be geographic differences of flora which honey is produced.

Can et al., reported that physicochemical and bioactive properties of honey are affected by flora and geographical differences [14]. Actually antioxidant property is the defense mechanism of plants against environmental factors such as climatic conditions. UV radiation, temperature, water stress, mineral nutrient presence were known the most important ones among these environmental factors.

Hence, the antioxidant activity of honey may differs which has the same botanical origin but produced in different regions [19]. Also it was reported that humidity and soil composition effect the variability of antioxidant capacity [5].

In general, dark coloured honey samples had higher antioxidant activity than the light coloured honey samples [20]. Likewise, we determined that oak honey was the darkest honey and has the highest antioxidant activity. It is known that the color reflects the content of pigments present in honey. Therefore, amber and dark amber honey should have higher total phenolic content than light amber honey [13]. The differences in colors was because of botanical origin and amount of suspended particles such as pollen [21]. The increase of honey color intensity is related to the increase in antioxidant properties and in phenolic content [22]. Although the darkest honey was flower honey after oak honey, flower honey is not ranked 2nd in the highest antioxidant capacity ranking. This can be explained by the difference in bioactive components in honey samples. Soares et.al. reported that antioxidant activity is more associated with the bioactive compound content than the color [19].

4. Conclusion

As a result, it is a wiser method to use natural bee products such as honey instead of using antibiotics and is important to use it in medicine and pharmaceutical industry. Honeys under analysis may have a relevant role as antibacterial natural products to decrease the effects of bacterial infections and contribute for better food. From the previous studies it is known that one of the factors affecting the quality of honey is antioxidant capacity. Therefore, it is recommended to consume honey with high antioxidant value in daily nutrition.

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