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Research Article

DETERMINATION THE ANTIBACTERIAL PROPERTIES OF HONEY

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ABSTRACT. Nowadays, the number of bacteria that gain resistance against antibiotics is increasing and resistant new strains against these drugs are forming. Researchers are searching for alternative medicines to solve this problem and are working on natural products. The aim of this study was to investigate the effects of honey that have shown antimicrobial activity on bacterial strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 which cause various diseases. The antimicrobial activity of sunflower honey and chestnut honey prepared in different concentrations collected from Trakya and Kocaeli regions were investigated and the disc diffusion method was used for the test in this study. As a result, while sunflower honey did not show antimicrobial activity against two subspecies, chestnut honey showed antimicrobial activity against both subspecies.

Keywords: *antimicrobial activity, honey, Escherichia coli, Staphylococcus aureus*

INTRODUCTION

Bacteria are beginning to gain resistance to antibiotics and new strains resistant to antibiotics are emerging [1]. In order to solve such problems, researchers continuously search for and synthesize new drugs. However, the cost of such drugs is high and production is less [2]. Thus, in most countries, researchers are reorienting to alternative treatment methods, allowing natural, cost-effective, and antimicrobial products to be used. Nowadays, expanding the studies applied to alternative and natural treatments and solving the diseases are benefiting humanity and nature [3]. In most countries, therapy, which is a natural treatment, uses the products that the bees produce. As honey is among the most commonly used foods with the greatest antimicrobial efficacy in the treatment, interest in the medical use of honey is increasing [4]. Julie et al. [5] showed that honey has some roles in the clinical treatment of infectious diseases. Katrina and Calvin [6] stated that honey performed antimicrobial activity against a broad spectrum of both gram-negative and positive bacteria. In addition to this, Mansour [7] showed that honey affected both vegetative and spore forms of *Clostridium botulinum.*

Honey is a natural product of honey bees and these bees are found associated with forests globally. The forest flowers ensure substances to honey bees in order to produce honey. Also, the forestry trees provide space for a bee-hive in which the honey bees live and produce honey [8]. Recent studies emphasize the importance of agroforestry for honey production [9].

The purpose of this study is to investigate the antimicrobial activity of honey produced from sunflower and chestnut trees. The test organisms used in this study were *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) strains which cause various diseases.

The objective of this study is to determine the antimicrobial effect of honey, produced from sunflower and Chesnut nectars and pollens by honeybees, on bacterial strains in order to test the usability of these honey for the treatment of infectious diseases. *E. coli* is a gram-negative bacteria and a part of a normal intestinal flora but in some cases, this bacterium causes some infections. Recently, Cools [10]reported that *E. coli* is a dominant bacterium caused by pregnancy-related maternal and neonatal morbidity and mortality. *S. aureus* is a gram-positive bacteria and defined as both commensal and also pathogen in humans. This bacterium causes bacteremia, infective endocarditis and also some infections in the skin [11]. This study focuses on the determination of the antibacterial effect of different honey types against *E. coli* and *S. aureus* which are opportunistic pathogens for a human.

MATERIALS AND METHODS

Honey, Bacteria, and Media used

Two types of honey were used, namely sunflower honey (SFH) and chestnut honey (CH). SFH and CH were obtained from Trakya and Kocaeli Regions in Turkey, respectively. Sunflower is an allogenic plant and needs insects like honey bees for the flowering stage [12]. These honeybees collect the nectar and pollens of the sunflowers in order to produce honey. The chestnut tree is one of the best sources for nectar and pollen at the beginning of summer for bees [13]. The honey samples were stored in dark bottles away from sunlight in the laboratory at +4°C.Bacterial strains *Escherichia coli* ATCC 05922 ve *Staphylococcus aureus* ATCC 29213 were used in this study. These two strains are recommended reference strains for antimicrobial activity tests [14]. The content of media used in this study is listed as; Nutrient Broth: Gelatin Peptone 5.00 g/L; Beef Extract 3.00g/L and üeller Hinton Agar: Meat infusion 2.00 g/L; Casein hydrolysate 17.50 g/L; Starch 1.50 g/L; Agar-agar 13.00 g/L.

Preparation of Honey Samples

To examine the effects of different concentrations of honey samples used for the determination of antimicrobial activity on the growth of bacterial strains, honey concentrations were prepared according to the concentrations given in Table 1. The honey samples were diluted with a sterile physiological saline solution (PSS).

Table 1. *Concentrations of honey for sunflower (SFH) and chestnut (CH) diluted with a physiological saline solution (PSS)*

Honey Concentration (%)	The amount of Honey (mL))	The amount of PSS (mL)
25	2.5	7.5
50	5.0	5.0
75	7.5	2.5
100	10.0	0

Kirby-Bauer Disk Diffusion Method

Each of the bacteria to be used in the study was inoculated into Nutrient Broth stocks contained in 50 ml aliquots and incubated at 37°C for 24 hours in a shaking incubator. At the end of the incubation period, bacterial suspensions were prepared from bacterial cultures, at a McFarland 0.5 concentration ($1.5X10^8$ CFU/mL). The previously sterilized Mueller Hinton agar (24 mL) media were poured into the Petri dishes. Under sterile conditions, wells with 5 mm in diameter were opened in the solid Mueller Hinton agar medium and half solid agar was dropped into the wells to cover the bottom area. 200 µL of the bacterial suspension, prepared according to the McFarland value, was inoculated by petri dish spreading. After inoculation, 250 µL of honey samples with different concentrations were loaded into each well and, then the inoculated Petri dishes containing honey were incubated at 37°C for 24 hours. Also inoculated Petri dishes without honey samples were used as the control group in the same conditions. The inhibition zones formed at the end of the incubation period were measured in mm and less than 12 mm zone was considered to have no adequate antimicrobial activity [15].

RESULTS AND DISCUSSION

The results of the antimicrobial activity of sunflower (SFH) and chestnut (CH) honey against the bacteria *Escherichia coli* ATCC 05922 and *Staphylococcus aureus* ATCC 29213 are given in Table 2.

Honey samples with different concentrations were used to determine antibacterial activity against *Escherichia coli* ATCC 059222 and *Staphylococcus aureus* ATCC 29213. As seen in Table 1, sunflower honey samples at all concentrations did not show any antibacterial effect against both of the two strains, while chestnut honey samples showed significant inhibitory activity against bacterial growth of both strains at the concentrations of 50, 75 and 100%.

Table 2. *Results of antimicrobial activity of honey samples (SFH: Sunflower honey; CH: Chestnut honey)*

The type of Honey	The concentration of Honey (%)	*Escherichia coli* ATCC 05922	*Staphylococcus aureus* ATCC 29213
SFH	25	-	-
SFH	50	-	-
SFH	75	-	-
SFH	100 (pure)	-	-
CH	25	-	-
CH	50	+	+
CH	70	+	+
CH	100 (pure)	+	+

-: The inhibition zone could not be determined; +: The diameter of the inhibition zone (mm)

Fig. 1 and 2 show the photographs of the results of antimicrobial tests of SFH and CH (100% concentration), respectively. Although the inhibition zone was not significant in

the presence of SFH (Fig. 1), the inhibition zones clearly seemed in the presence of CH (Fig. 2). The occurrence of transparent inhibition zone formation in the Petri dishes containing sunflower honey (Fig. 1) was considered that sunflower honey has a bacteriostatic effect instead of the bactericidal effect. The significant inhibition zones in Petri dishes with chestnut honey indicated the bactericidal activity (Fig. 2).

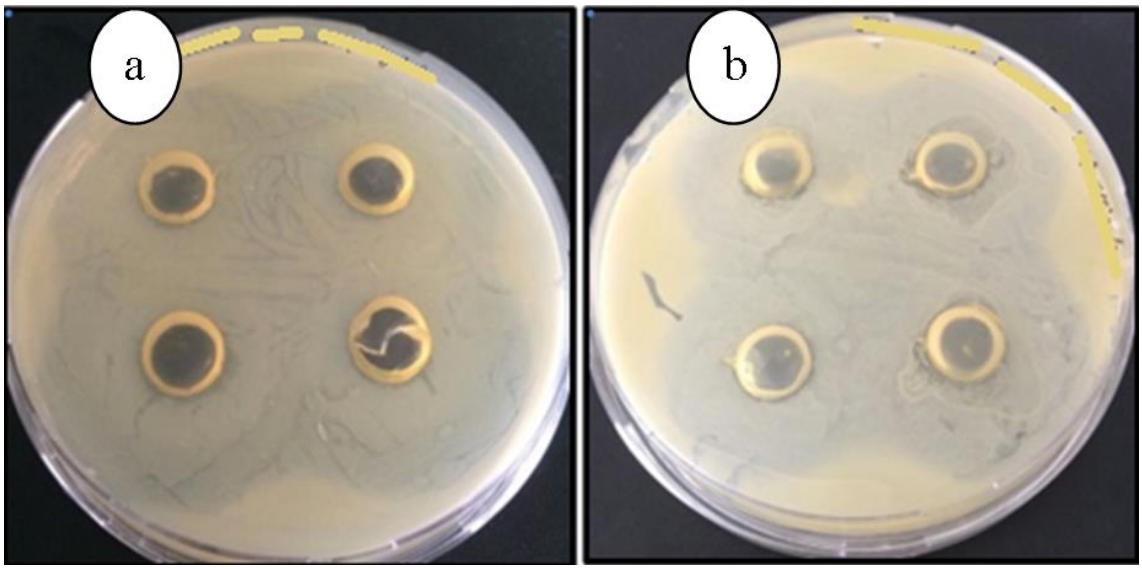

Fig. 1. *The antimicrobial effect of sunflower honey against E. coli ATCC 059222 (a) and S. aureus ATCC 29213(b)*

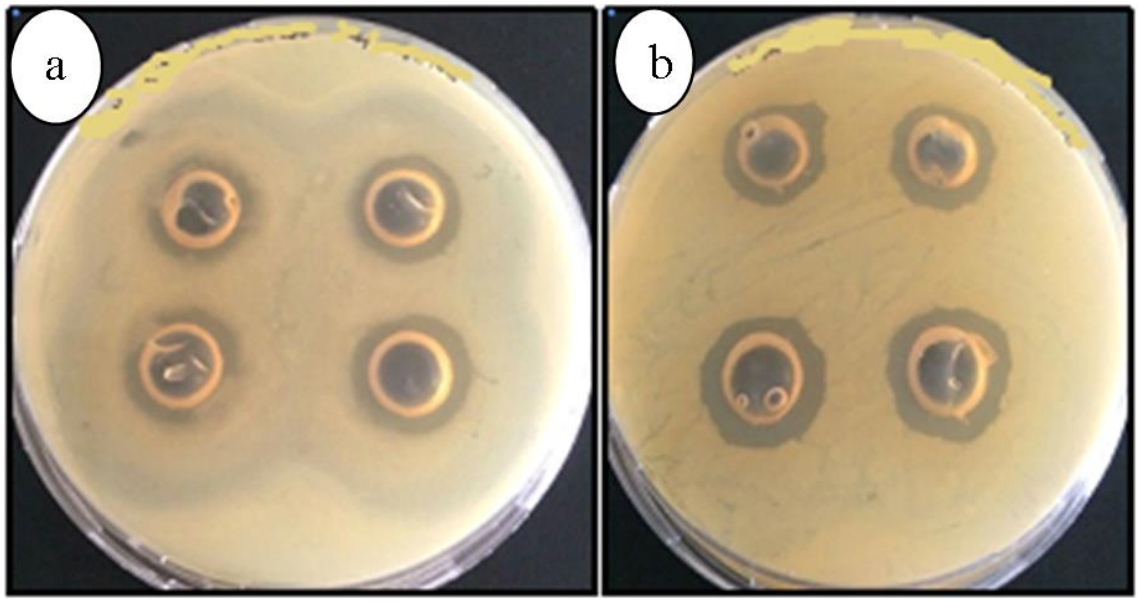

Fig. 2. *The antimicrobial effect of chestnut honey against E. coli ATCC 059222 (a) and S. aureus ATCC 29213(b)*

The diameters of inhibition zones of chestnut honey concentrations are shown in Fig. 3. The antibacterial activity of pure (100%) chestnut honey and 75% chestnut honey showed the highest antibacterial activity against *Staphylococcus aureus* ATCC 29213 (19 mm inhibition zone) while the least antibacterial activity was 50% chestnut honey against

Escherichia coli ATCC 059222 (16 mm inhibition zone). 25% of chestnut honey did not show any antibacterial effect against both of the strains.

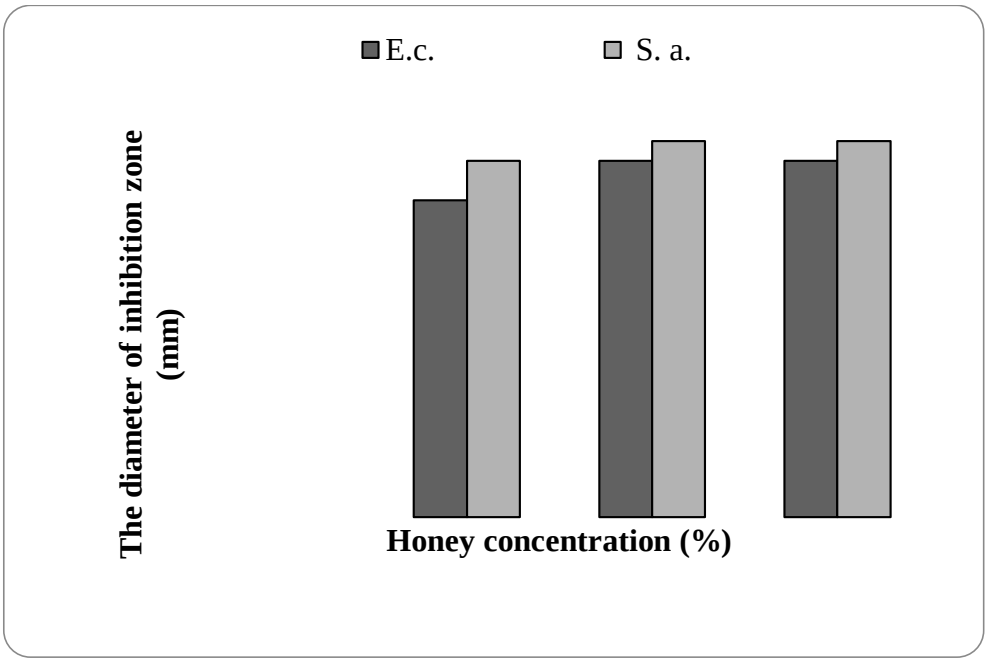

Fig. 3. *The inhibition zone graph at different chestnut honey concentrations*

The antimicrobial activity of different kinds of honey have been proven by some recent studies in literature and these studies concluded that honey killed most of the pathogenic microorganisms [16]. The results of this study show that chestnut honey performed antimicrobial activity against the bacterial strains used in this study. On the other hand, the sunflower honey did not have any antimicrobial performance. It is considered that the components of these different honey types were different and these components have a significant role in their activity. It is known that the antimicrobial effect of honey is caused by hydrogen peroxide, flavonoids, which is in high molarity, low humidity, and acidic character, as well as in its structure [17]. The chemical components of honey such as benzoic acid, cinnamic acid, and flavonoids have been assayed to have an antibiotic effect on microorganisms [18]. Mundo et al. [19]reported that mold, yeast and bacterium spores could be found at low levels, but vegetative bacteria forms were not commonly found. At the same time, these investigators showed that *Bacillus stearothermophilus* as the most susceptible microorganism to antimicrobial activity and *Staphylococcus aureus, Penicillium expansium, Aspergillus niger* and *Geotrichum candidum* as the least affected microorganisms. And also *Staphylococcus aureus* ATCC 25923, 8095 and 9144 are susceptible to the antimicrobial activity of honey. The growth of *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus* ATCC 8095 have been reduced due to the high osmotic pressure of honey. Estevinho et al. [20] reported that the extract of the phenolic compounds of northeastern Portuguese honey has antimicrobial activities against Gram (+) bacteria (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus lentus*) and Gram (-) bacteria (*Pseudomonas aeuruginosa, Klebsiella pneumonia, and Escherichia coli*). Also, the same researchers showed that *S. aureus* was the most sensitive microorganism, whereas *B. subtilis, S. lentus, K. pneumon*ia, and *E. coli* were moderately sensitive bacteria. In this study the antimicrobial activity of two types of

honey such as sunflower and chestnut against gram (+) bacterium named *Staphylococcus aureus* ATCC 29213 and Gram (-) bacterium called *Escherichia coli* ATCC 059222. The sunflower honey did not show any antimicrobial activity, but chestnut honey showed antimicrobial activity. It was found that *Staphylococcus aureus* ATCC 29213 was more sensitive than *Escherichia coli* ATCC 059222. As it is known that the cell wall of gram-negative bacteria is complex and has a thin peptidoglycan layer and an outer layer, the cell wall of gram-positive bacteria has a simpler structure and has a thick peptidoglycan layer. It was expected that the honey would have more effect on bacterial strains with simple cell walls than bacteria with these two different structures. Because the grams staining technique is the most damaging of the walls and the chemical staining areas are gram-positive bacteria, whereas the gram-negative bacteria have the dye-holding ability when treated with a second chemical dye. In addition to this information, gram-negative bacteria are more pathogenic and cause many diseases in humans, which is due to the cell wall properties of gram-negative bacteria [21]. The results of this study support that gram-negative bacteria had more resistance against honey than gram-positive bacteria. On the other hand, the killer effect of chestnut honey against *E. coli,* which was a pathogenic featured gram-negative bacteria, was observed in this study. Hazır and Keskin [22] have studied with the bacterial strains called *Staphylococcus aureus, Escherichia coli O157: H7, Klebsiella pneumonia* in their study and reported that increasing honey concentrations increased the diameters of inhibition zones formed on bacteria. Similarly, in this study, it was determined that the increment of chestnut honey concentrations resulted in the increase of inhibition zone diameters.

The antimicrobial activity of honey is a very complex process and the mechanism has not explained clearly yet. The studies published in the literature reported that varies components of the honey played a significant role in its antimicrobial activity [23-28].One of the explanations about the antimicrobial activity of honey is related with the high sugar concentration of the honey, which prevents the growth of microorganisms such as bacteria without having the tolerance to osmotic pressure [23]. Another explanation for the antimicrobial property of honey is revealed to the low pH value of the honey and also containing a high concentration of organic acids like gluconic acid. Honey contains a peptide called bee defensin-1, which was secreted from the hypopharyngeal glands of the honey bee. It is considered that the bee defensin-1 had an important role in the health care of bee larvae due to its activity against gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Paenibacillus larvae*. It is known that the high sugar concentration and low pH content of honey are common antimicrobial properties of all honey types, but the amount of the peptide called bee defensin- 1 (also named royal jelly) differentiate the antimicrobial property of the honey types [28]. It is also reported that bees produce other types of peptides in order to care for their immune system from bacterial diseases, but these peptides were not detected in the component of honey [27]. An oxidoreductase enzyme called glucose oxidase catalyzes the the oxidation reaction of glucose to gluconic acid. While the oxidation reactions occurred the hydrogen peroxide (H_2O_2) was formed as a side product showing the high antimicrobial property. The glucose oxidase is secreted from the honeybees' salivary glands to affect the collected nectar and protects the honey from microorganisms especially pathogenic ones. It is interesting that this present enzyme has not any activity in the maturing process of the honey. The results of this study showed that different kinds of honey did not perform the same antimicrobial activity. The kinds of plant resources, which were used for honey production, significantly affected the antimicrobial activity of the product.

CONCLUSION

The antimicrobial activities of sunflower and chestnut honey against a gram-negative and gram-positive bacteria were determined in this study. In the results of antimicrobial activity tests, chestnut honey was found to have the most bactericidal effect against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 059222 strains. It has been observed that sunflower honey does not have a bactericidal effect against these two strains but has an inhibitory effect on their growth as a bacteriostatic effect. From these data, antimicrobial activity compounds obtained from honey can be used in the pharmaceutical industry in order to develop new antimicrobial drugs to treat bacterial infections.

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