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Research Article

Antimicrobial Effect of Honeys Collected in Bingol Region

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Abstract

In this research, the antimicrobial effects of honeys collected from Genç, Kiğı, Sancak, and Yedisu districts of Bingöl province were investigated by disc diffusion method. The antimicrobial effects of honey samples prepared at three different concentrations (500, 250, and 125 mg mL^{-1}) were tested using Staphylococcus aureus ATCC 29213 and Listeria monocytogenes NCTC 5348 bacteria as Gram positive (+), Escherichia coli ATCC 25922 bacterium as Gram negative (-), Saccharomyces cerevisiae ATCC 76521 as yeast, and Candida albicans ATCC 90028 as fungus (mold). In addition, Ampicillin/Sulbactam (SAM) (20 µg/disc) was used as an antibiotic to better evaluate the antimicrobial effects of honeys in this research. The antimicrobial effect of Ampicillin/Sulbactam (SAM) (20 µg $disc^{-1}$) against the microorganisms used in honey samples was also tested with the same method.As a result; while the 500 and 250 mg mL^{-1} concentrations among honey samples prepared at three different concentrations (500, 250, and 125 mg mL^{-1}) have an antibacterial effect against Staphylococcus aureus, the antibacterial effect of the concentrations of 125 mg mL^{-1} against Staphylococcus aureus was not detected. All honey samples at three different concentrations showed no antibacterial effect against Listeria monocytogenes. While only the 500 mg mL^{-1} concentrations from different concentrations of Genç and Yedisu honey samples were found to have an antibacterial effect against Escherichia coli, the antibacterial effects of Kiğı and Sancak honey samples prepared at different concentrations against Escherichia coli were not detected. It was determined that only 500 mg mL^{-1} concentrations from all honey samples had an antimicrobial effect against Saccharomyces cerevisiae, while the other 250 and 125 mg mL^{-1}concentrations did not have an antimicrobial effect against Saccharomyces cerevisiae. The studied concentrations of all honey samples did not show an antifungal effect against Candida albicans. Moreover, Ampicillin/Sulbactam (SAM) (20 µg $disc^{-1}$) was found to have a high antimicrobial effect against Staphylococcus aureus and Listeria monocytogenes (Gram-positive bacteria), Escherichia coli (Gram-negative bacteria), Saccharomyces cerevisiae (yeast), and Candida albicans (fungus) microorganisms.

Keywords: Bingöl, honey, antibacterial effect, antifungal effect, antimicrobial effect

INTRODUCTION

Honey is one of the oldest traditional foods known for its antimicrobial effect for thousands of years and has been used effectively in the treatment of burns and wounds as well as a remedy for microbial infections (Zumla and Lulat, 1989; Brudzynski, 2006). Honey owes its antimicrobial effect to hydrogen peroxide, strong osmotic effect, acidity, aromatic acids, flavonoids, phenolic compounds, lysozyme, and various phytochemicals (Molan, 1992a, b; Libonatti et al., 2014; Kačániová et al., 2011 and Wahdam, 1998). Estrada et al. (2005) also reported that honey is a well-known antibacterial agent in their study. Honey contains more than 150 phenolic compounds that are effective against a variety of Gram-positive and Gram-negative bacteria and have potent antioxidant properties (Davidson, 1993). Molan (1992a) and Çenet (2019) reported that honey has strong antifungal effect and can be used as an important antifungal agent due to this feature. Çenet et al. (2015) and Çenet (2019) proved that honey samples from Kahramanmaraş and the west part of Turkey inhibited microbial growth of *Bacillus megaterium*, *Baccilus subtilis*, *Staphylococcus aureus*, *Candida albicans* in their performed study. In other studies by Cooper et al. (2002) and Willix et al. (1992), it was reported that honey has antibacterial effect against pathogenic bacteria such as *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*. The aim of the present study was to determine and compare the antimicrobial effects of honey samples collected from different districts (Genç, Kiğı, Sancak, and Yedisu) of Bingöl province (Turkey) against *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* NCTC 5348, *Escherichia coli* ATCC 25922, *Saccharomyces cerevisiae* ATCC 76521, and *Candida albicans* ATCC 90028 microorganisms by disc diffusion assay. Ampicillin/Sulbactam (20 µg/disc) was also used as an antibiotic to better evaluate the antimicrobial effects of honey, and it demonstrated different antimicrobial effects on these microorganisms.

MATERIALS and METHODS

Materials

Honeys, sterile ddH_2O, Mueller-Hinton (MH) agar (MERCK 103872), Bioanalyse antimicrobial susceptibility testing discs as blank discs, and Ampicillin/Sulbactam (SAM) (20 µg/disc) antibiotic discs.

Honeys

Honeys were procured from beekeepers of those in Genç, Kiğı, Sancak (in Bingöl center), and Yedisu districts of Bingöl province, Turkey (38° 44' 54.2580" N - 40° 33' 14.1552" E, 38° 53' 7.6704" N - 40° 29' 47.8464" E, 39° 26' 2.2020" N - 40° 32' 43.0512" E, 39° 5' 42.7200" N - 40° 24' 5.8680" E, respectively.) in 2022 (Fig. 1). The collected honeys were stored at 25 °C in the dark until the honeys were tested by disc diffusion assay.

Figure 1. A map of the districts of Bingöl province giving the approximate location of procured honey samples

Preparation of Honey Samples

Honey samples were prepared in sterile double distilled water (ddH_2O) at the three different concentrations of 500, 250, and 125 mg/mL, respectively. Namely, 0.5, 0.25, and 0.125 g from each of those of Genç, Kiğı, Sancak, and Yedisu honey samples were weighed. 1 mL sterile ddH_2O was added to each of them, then the samples were dissolved well in sterile ddH_2O by using an ultrasonic water bath. After that, the 40 µL of each of prepared honey sample was impregnated on the sterile blank paper discs (6 mm diameter, 3mm thickness).

Microorganisms

In this study, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* NCTC 5348 (Gram-positive (+) bacteria), *Escherichia coli* ATCC 25922 (Gram-negative (-) bacteria), *Saccharomyces cerevisiae* ATCC 76521 (yeast), and *Candida albicans* ATCC 90028 (fungus) were used to test antimicrobial effect of honey samples.

Antimicrobial Effect

Disk diffusion method was performed to determine the antimicrobial effects of honey samples against microorganisms described in section 2.4. Briefly, test microorganisms at a concentration of 40 microliters 0.5 McFarland (1.5 x 10^8 microorganisms/mL) were inoculated on Mueller-Hinton agar plates. Then, the discs containing honey samples, and SAM (20 µg/disc) containing antibiotics were placed on the surface of inoculated agar plates. In order to observe the antimicrobial zone inhibitions caused by honey samples, the prepared Petri plates were kept at 37 °C for 24 hours (Radji et al., 2013).

Statistical Analysis

All measurements were done in triplicate, and the average values of zone

inhibition diameters were stated as mean ± SD.

RESULTS and DISCUSSION

Antimicrobial Effect

The antimicrobial effect values (the zone inhibition diameters) of three different concentrations (500, 250, and 125 mg/mL) of different local (Genç, Kiğı, Sancak, and Yedisu) honey samples produced in Bingöl (Turkey) are presented in Table 1. Table 1 indicates the antimicrobial effect results (the zone inhibition diameters) in three different concentrations (500, 250, and 125 mg/mL) of different local (Genç, Kiğı, Sancak, and Yedisu) Bingöl honey samples against *S. aureus* ATCC 29213, *L. monocytogenes* NCTC 5348, *E. coli* ATCC 25922, *S. cerevisiae* ATCC 76521, and *C. albicans* ATCC 90028 by disc diffusion assay. Also, SAM (20 µg/disc) showed different antimicrobial effects on these microorganisms. The zone inhibition diameters of Genç, Kiğı, Sancak, and Yedisu honey samples for 500 mg/mL concentration against *S. aureus* were determined as 9.5-9.0-8.5-8.0 mm, respectively. For 250 mg/mL concentration of honeys, the zone inhibition diameters against *S. aureus* were determined as 7.0-6.0-6.5-6.0 mm, respectively. For 125 mg/mL concentration of honeys, the zone inhibition diameters against *S. aureus* were not at detectable levels. The zone inhibition diameters of SAM against *S. aureus* were found as 15-14-14-11.5 mm, respectively (Table 1). The Genç, Kiğı, Sancak, and Yedisu honey samples have antimicrobial effect against *S. aureus* strain. *S. aureus* strain was the most sensitive strain against the Genç, Kiğı, Sancak, and Yedisu honey samples (Table 1). The antimicrobial effect values (the zone inhibition diameters) against *S. aureus* of the Genç, Kiğı, Sancak, and Yedisu honey samples were lower than the antimicrobial effect values (the zone inhibition diameters) found for *S. aureus* by Nzeako and Hamdi (2000) for Germany and Turkey honeys, Fahim et al. (2014) for Pakistan honeys, Osho and Bello (2010) for honeys produced by the *Apis mellifera*, Abd-El Aal et al. (2007) for Egypt honeys, Grego et al. (2016) for Piedmont honeys, Dryden et al. (2014) for Surgihoney, Nedie Patience et al. (2020) for Nigeria honeys, were higher than the antimicrobial effect values found for *S. aureus* by Šedík et al. (2018) for Slovak honeys, Gulfraz et al. (2010) for various honey types of Pakistan, were in agreement with the antimicrobial effect values determined for *S. aureus* by Kaya and Yıldırım (2021) for Bingöl honeys, Çakır et al. (2020) for honeys of Rize, Gümüşhane, and Sivas provinces. The zone inhibition diameters of Genç, Kiğı, Sancak, and Yedisu honey samples for 500, 250, and 125 mg/mL concentrations against *L. monocytogenes* were not at detectable levels. The zone inhibition diameters of SAM against *L. monocytogenes* were determined as 36.0-35.5-37.0-36.5 mm, respectively (Table 1). The Genç, Kiğı, Sancak, and Yedisu honey samples have no antimicrobial effect against the *L. monocytogenes* strain. *L. monocytogenes*, together with *C. albicans* were the most resistant strains against Genç, Kiğı, Sancak, and Yedisu honey samples (Table 1). The antimicrobial effect values determined against *L. monocytogenes* of Genç, Kiğı, Sancak, and Yedisu honey samples were lower than the antimicrobial effect values found for *L. monocytogenes* by Šedík et al. (2018) for Slovak honeys, were compatible with the antimicrobial effect values presented for *L. monocytogenes* by Çakır et al. (2020) for honeys of Rize, Gümüşhane, and Sivas provinces. The zone inhibition diameters of Genç and Yedisu honey samples for

500 mg/mL concentration against *E. coli* were found as 6.0-6.0 mm, respectively. The zone inhibition diameters of Kiğı and Sancak honey samples for 500 mg/mL concentration against *E. coli* were not at detectable levels. For 250 and 125 mg/mL concentrations of all honey, the zone inhibition diameters against *E. coli* were not at detectable levels. The zone inhibition diameters of SAM against *E. coli* were determined as 11.0-8.5-6.5-12.0 mm, respectively. (Table 1). The Genç and Yedisu honey samples have an antimicrobial effect against the *E. coli* strain, whereas Kiğı and Sancak honey samples have no antimicrobial effect against the *E. coli* strain. *L. monocytogenes* and *C. albicans* strains were the most resistant strains against the Genç, Kiğı, Sancak, and Yedisu honey samples, followed by the *E. coli* strain (Table 1). The antimicrobial effect values determined against *E. coli* of Genç, Kiğı, Sancak, and Yedisu honey samples were lower than the antimicrobial effect values reported for *E. coli* by Nzeako and Hamdi (2000) for Germany and Turkey honeys, Garedew et al. (2003) for *Trigona* bee honey, Fahim et al. (2014) for Pakistan honeys, Osho and Bello (2010) for honeys produced by the Apis mellifera, Šedík et al. (2018) for Slovak honeys, Grego et al. (2016) for Piedmont honeys, Dryden et al. (2014) for Surgihoney, Nedie Patience et al. (2020) for Nigeria honeys, Ghramh et al. (2020) for Saudi Arabia and Pakistani honeys, Kaya and Yıldırım (2021) for Bingöl honeys, were higher than the antimicrobial effect values found for *E. coli* by Gulfraz et al. (2010) for various honey types of Pakistan, were in agreement with the antimicrobial effect values determined for *E. coli* by Çakır et al. (2020) for honeys of Rize, Gümüşhane, and Sivas provinces. The zone inhibition diameters of Genç, Kiğı, Sancak, and Yedisu honey samples for 500 mg/mL concentration against *S. cerevisiae* were determined as 8.5-8.0-6.0-6.0 mm, respectively. For 250 and 125 mg/mL concentrations of all honey, the zone inhibition diameters against *S. cerevisiae* were not at detectable levels. The zone inhibition diameters of SAM against *S. cerevisiae* were determined as 11.5-12.5-9.0-9.5 mm, respectively (Table 1). The Genç, Kiğı, Sancak, and Yedisu honey samples have an antimicrobial effect against the *S. cerevisiae* strain. *S. aureus* strain was the most sensitive strain against the Genç, Kiğı, Sancak, and Yedisu honey samples, followed by the *S. cerevisiae* strain (Table 1). The antimicrobial effect values determined against *S. cerevisae* of all honey samples in this study were higher than the antimicrobial effect values found for *S. cerevisiae* by Garedew et al. (2003) for *Trigona* bee honey, were similar to the antimicrobial effect values found for *S. cerevisiae* by Kaya and Yıldırım (2021) for Bingöl honeys, Çakır et al. (2020) for honeys of Rize, Gümüşhane, and Sivas provinces. The zone inhibition diameters of Genç, Kiğı, Sancak, and Yedisu honey samples for 500, 250, and 125mg/mL concentrations against *C. albicans* were not at detectable levels. The zone inhibition diameters of SAM against *C. albicans* were determined as 29.0-31.0-29.0-27.0 mm, respectively (Table 1). The Genç, Kiğı, Sancak, and Yedisu honey samples have no antimicrobial effect against the *C. albicans* strain. *C. albicans*, together with *L. monocytogenes* were the most resistant strains against Genç, Kiğı, Sancak, and Yedisu honey samples (Table 1). The zone inhibition diameters determined against *C. albicans* of honey samples in this study were lower than the zone inhibition diameters reported for *C. albicans* by Nzeako and Hamdi (2000) for Germany and Turkey honeys, Fahim

et al. (2014) for Pakistan honeys, Gulfraz et al. (2010) for various honey types of Pakistan, Dryden et al. (2014) for Surgihoney, Ghramh et al. (2020) for Saudi Arabia and Pakistani honeys, were in agreement with the zone inhibition diameters reported for *C. albicans* by Kaya and Yıldırım (2021) for Bingöl honeys, Çakır et al. (2020) for honeys of Rize, Gümüşhane, and Sivas provinces.

Table 1. The antimicrobial effect values (the zone inhibition diameters) of three different concentrations (500, 250, and 125 mg/mL) of different local (Genç, Kiğı, Sancak, and Yedisu) honey samples produced in Bingöl (Turkey)

Tested microrganism	Genç				Kiğı				Sancak				Yedisu			
	Zone of inhibition (mm)[a]															
	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM
Gram (+)																
Staphylococcus aureus	9.5 ± 0.7	7.0 ± 0.0	-	15.0 ± 1.4	9.0 ± 0.0	6.0 ± 0.0	-	14.0 ± 1.4	8.5 ± 0.7	6.5 ± 0.7	-	14.0 ± 1.4	8.0 ± 0.0	6.0 ± 0.0	-	11.5 ± 0.7
Listeria monocytogenes	-	-	-	36.0 ± 1.4	-	-	-	35.5 ± 2.1	-	-	-	37.0 ± 1.4	-	-	-	36.5 ± 2.1
Gram (-)																
Escherichia coli	6.0 ± 0.0	-	-	11.0 ± 1.4	-	-	-	8.5 ± 0.7	-	-	-	6.5 ± 0.7	6.0 ± 0.0	-	-	12.0 ± 1.4
Yeast																
Saccharomyces cerevisiae	8.5 ± 0.7	-	-	11.5 ± 0.7	8.0 ± 1.4	-	-	12.5 ± 0.7	6.0 ± 0.0	-	-	9.0 ± 1.4	6.0 ± 0.0	-	-	9.5 ± 0.7
Fungus																
Candida albicans	-	-	-	29.0 ± 4.2	-	-	-	31.0 ± 1.4	-	-	-	29.0 ± 1.4	-	-	-	27.0 ± 1.4

–: No effect
a Values are the average of triplicate and expressed as mean ± SD
b Honey concentrations (mg/mL); SAM, Ampicillin/Sulbactam (20 µg/disc)

CONCLUSIONS

Table 1 indicates the zone inhibition diameter results in three different concentrations (500, 250, and 125 mg/mL) of different local (Genç, Kiğı, Sancak, and Yedisu) Bingöl honey samples against *S. aureus* ATCC 29213, *L. monocytogenes* NCTC 5348, *E. coli* ATCC 25922, *S. cerevisiae* ATCC 76521, and *C. albicans* ATCC 90028 by disc diffusion assay. Also, SAM (20 µg/disc) was used as an antibiotic to better evaluate the antimicrobial effects of honey, and it demonstrated different antimicrobial effects on these microorganisms. Genç, Kiğı, Sancak, and Yedisu honey samples at 125 mg/mL concentration, showed no antimicrobial effect against *S. aureus, L. monocytogenes, E. coli, S. cerevisiae*, and *C. albicans*, whereas these honey samples at 250 mg/mL concentration showed antimicrobial effect against only *S. aureus*. Genç, Kiğı, Sancak, and Yedisu honey samples at 500 mg/mL concentration, exhibited inhibitions against *S. aureus* and *S. cerevisiae*, while they did not show any antimicrobial effect against *L. monocytogenes* and *C. albicans*. Also, Genç and Yedisu honey samples at 500 mg/mL concentration, exhibited mild antimicrobial effect against *E. coli*, while Kiğı and Sancak honey samples at 500 mg/mL

concentration did not show antimicrobial effect against *E. coli*. All honey samples at three different concentrations showed no antimicrobial effect against *L. monocytogenes* and *C. albicans*. Moreover, SAM (20 µg/disc) was found to have a high antimicrobial effect against *S. Aureus*, *L. monocytogenes*, *E. coli*, *S. cerevisiae*, and *C. albicans*. For the all honey samples in this study, *C. albicans* and *L. monocytogenes* were the most resistant strains followed by *E. coli* and *S. cerevisiae* strains, respectively. Together with that *S. aureus* was the most sensitive strain. The results have shown that the Genç honey sample has the maximum antimicrobial effect followed by Kiğı, Sancak, and Yedisu honey samples, respectively. Moreover, SAM (20 µg/disc) has the highest antimicrobial effect against *L. monocytogenes* and *C. albicans* strains followed by *S. aureus* strain, whereas it has the lowest antimicrobial effect against *E. coli* and *S. cerevisiae* strains, respectively.

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