ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi*

DOI: 10.5152/AUAF.2023.23148

Comparison of Antimicrobial Activities of Ethanol- and Water-Based Propolis Extracts on Various Foodborne Pathogens by Agar-Well Diffusion Method

Etanol ve Su Bazlı Propolis Ekstraktlarının Çeşitli Gıda Kaynaklı Patojenler Üzerindeki Antimikrobiyal Aktivitelerinin Agar-Well Difüzyon Yöntemi ile Karşılaştırılması

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ABSTRACT

Although ethanolic-based propolis extracts have been shown to have various biological activities, studies on the differences in the antimicrobial effects of water- and ethanol-based extracts, are lacking. Propolis preparations are now also sold as water-based products due to a number of factors, such as being safer for babies, being biocompatible, and some religious prohibitions on alcohol consumption. This study aimed to compare the antimicrobial effects of commercially available propolis preparations in the form of ethanol- and water-based extracts against various pathogenic microorganisms that cause foodborne infections. A total of 36 Gram-positive and Gram-negative bacterial strains from 8 different species were used in the study. As a result of the statistical analysis, only ethanol-based propolis from Turkey was effective against enterococci, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* subsp. *spizizenii*, *Salmonella* spp., *Listeria monocytogenes*, *Acinetobacter baumannii,* and *Pseudomonas aeroginosa*, except *Escherichia coli*. It was concluded that the water-based extract did not have a significant inhibitory effect on the bacteria tested. It was determined that both ethanol- and water-based propolis tested in the study showed the most inhibitory effect on *Acinetobacter baumannii*. In addition, we can also conclude that ethanol-based propolis extract is more effective against Gram-positive bacteria than Gram-negative bacteria.

Keywords: Antimicrobial agents, antimicrobial resistance, food safety, foodborne infections, propolis

ÖZ

Etanol bazlı propolis ekstraktlarının çeşitli biyolojik aktivitelere sahip olduğu gösterilmiş olmasına rağmen, su ve etanol bazlı ekstraktların antimikrobiyal etkileri arasındaki farklılıklara ilişkin çalışmalar eksiktir. Propolis müstahzarları, bebekler için daha güvenli olması, biyouyumlu olması ve alkol tüketimiyle ilgili bazı dini yasaklar gibi bir dizi faktör nedeniyle artık su bazlı ürünler olarak da satılmaktadır. Bu çalışma, gıda kaynaklı infeksiyonlara neden olan çeşitli patojen mikroorganizmalara karşı etanol ve su bazlı ekstrakt formundaki ticari olarak temin edilebilen propolis preparatlarının antimikrobiyal etkilerinin karşılaştırılmasını amaçlamıştır. Çalışmada sekiz farklı soydan toplam 36 Gram pozitif ve Gram negatif bakteri suşu kullanılmıştır. İstatistiksel analizler sonucunda Türkiye'ye ait su ve alkol bazlı propolis ektraklarından sadece etanol bazlı propolisin *Escherichia coli* hariç, enterococci, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* subsp. *spizizenii*, *Salmonella* spp., *Listeria monocytogenes*, *Acinetobacter baumannii,* and *Pseudomonas aeroginosa* bakterileri üzerine antimikrobiyel etki oluşturduğu tespit edilmiştir. Su bazlı ekstraktın test edilen bakteriler üzerinde önemli bir inhibitör etkiye sahip olmadığı sonucuna varılmıştır. Çalışmada test edilen etanol ve su bazlı propolislerin her ikisinin de *Acinetobacter baumannii*

Received/Geliş Tarihi: 27.06.2023
Accepted/Kabul Tarihi: 15.08.2023
Publication Date/Yayın Tarihi: 11.09.2023

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Cite this article as: Onaran Acar, B., & Gülendağ, E. (2023). Comparison of antimicrobial activities of ethanol- and water-based propolis extracts on various foodborne pathogens by agar-well diffusion method. *Research in Agricultural Sciences*, 54(3), 130-136.

üzerinde en fazla inhibitör etki gösterdiği belirlenmiştir. Ayrıca, etanol bazlı propolis ekstraktının Gram-pozitif bakterilere karşı Gram-negatif bakteriler üzerine daha etkili olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Antimikrobiyal ajanlar, antimikrobiyal direnç, gıda güvenliği, gıda kaynaklı infeksiyonlar, propolis

Introduction

Foodborne infections are considered to be among the main causes of high mortality and morbidity worldwide (Pires et al., 2021). Many people get sick and die from treatable diseases, especially in undeveloped countries, due to a lack of hygiene standards and basic healthcare (Fung et al., 2018). Antibiotics are known as the first choice for the treatment of foodborne bacterial infections. They have a bactericidal (killing bacteria) or bacteriostatic (inhibiting the growth of bacteria) effect on microorganisms by interacting with a specific target in the bacteria (Hutchings et al., 2019). However, antibiotics have been associated with numerous side effects, such as allergic reactions, immunosuppression, and hypersensitivity. In addition, it is known that they can increase the chance of colonization of pathogens in the intestine by causing suppression of beneficial microorganisms living in the intestinal mucosa (Ramirez et al., 2020). One of the main complications caused by bacterial infections is their resistance to commonly used antibiotics (Morehead et al., 2018). Therefore, antibiotic resistance is considered one of the critical public health problems today. The vast majority of clinically relevant bacteria have become resistant to many of the antibiotics commonly used today (Huemer et al., 2020). Frightening statistics have emerged regarding this matter. It is estimated that approximately 35,000 people died in 2019 as a result of infection by an antibiotic-resistant organism in the United States alone (Centers for Disease Control and Prevention [CDC], 2019).

Today, new and alternative antimicrobials are needed to effectively treat infections. In addition, there is a need in the food industry for low-cost, reliable, and preferably natural antimicrobial agents to delay microbial growth in foods. For this reason, research on the use of natural products and the discovery of new products is increasing day by day (Pisoschi et al., 2018).

Well known in apitherapy and beekeeping, propolis is a natural resin-containing substance produced by honey bees from the buds and secretions of various plant species. Propolis is used in making honeycombs, sealing, and sterilizing beehives by mixing with enzymes and pollen produced by bees. In this way, it protects the colony from diseases and invaders and is generally effective in ensuring the cleanliness of the environment (Santos et al., 2020). The mentioned protective properties of propolis are achieved thanks to its antimicrobial properties (Pobiega et al., 2019; Przybyłek & Karpiński, 2019). Studies on the use of propolis as a natural product in the fight against harmful microorganisms are constantly increasing. However, data on the antimicrobial effect of this bee product appear to be quite scarce (Freitas et al., 2022; Hünler-Dönmez, 2021).

Many types of propolis are defined and classified according to their botanical origins, physicochemical properties, and geographical locations (Santos et al., 2020). In general, the content of propolis contains 10% pollen, 30% wax, and 55% resin and balsams, and it is reported that it consists of more than 300 molecules. Among these 300 compounds, aromatic aldehydes and alcohols, esters, presence of terpenes, phenolic esters, fatty and phenolic acids, flavonoids, sesquiterpenes, β-steroids, and naphthalene have already been identified (Huang et al., 2014).

Studies on propolis concentrate on ethanolic-based extracts as it is widely used in therapeutic applications. The antimicrobial (Massaro et al., 2015), antioxidant (Zhao et al., 2016), antiviral (Bankova et al., 2014), antiparasitic (da Silva et al., 2016), antitumor (Chan et al., 2013), immunomodulatory (Gao et al., 2014), anti-inflammatory (Hori et al., 2013) and hepatoprotective (Babatunde et al., 2015) properties of ethanolic-based propolis extracts have been demonstrated in various studies. The fact that propolis has such a wide range of biological properties shows that it has application potential in the development of propolis-based products for use in human and animal health.

Ethanol is one of the most often used solvents for extracting propolis (Postali et al., 2022; Touzani et al., 2021). Propolis preparations are now also sold as water-based products due to a number of factors, including the fact that they are safer for infants and biocompatible, as well as some religious prohibitions against alcohol consumption. No comprehensive study was found to determine and/or compare the antimicrobial effects of commercially available propolis preparations in the form of ethanol- and water-based extracts against various pathogenic microorganisms causing foodborne infections. Therefore, this study aimed to determine the antimicrobial effects of propolis extracts, which are sold in two forms as ethanol- and water-based, against various pathogenic microorganisms that cause foodborne infections. Our research contributes to the literature as it compares the antibacterial activity of propolis extracts made from ethanol and water, and it determines this effect using the simple agar-well diffusion method.

Methods

Propolis Extracts

Ready-to-use water-based (30%) and ethanol-based (30%) propoli extracts were supplied from markets in Turkey.

Bacterial Strains

Overall, 36 bacterial strains, including *Enterococcus faecalis* ATCC 29212, *E. hirae* ATCC 10541, different wild-type *vanA* ($n=2$) and *vanM* ($n=8$) positive Enterococci isolates, *Staphylococcus aureus* ATCC 29213, *S. aureus* NCTC 13626, *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, wild-type *S. aureus mecC*+, *S. aureus mecA*+, *S. aureus* SEC+, *S. aureus* SEE+, *S. aureus* SEB+, *S. aureus* PVL+, *S. aureus* SED+ isolates, *Listeria monocytogenes* ATCC 7644, wild-type *Bacillus cereus, B. spizizenii* ATCC 6633, were used in the study as Gram-positive cultures. *Salmonella* Kentucky, *Salmonella enterica* subsp. *enterica* serotype Pullorum NCTC 10705, wild type *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *Salmonella enterica* subsp. *enterica* serovar Gallinarum NCTC 13346, wild type *Salmonella enterica* serovar Typhi, wild type *Salmonella enterica* subsp. *enterica* serovar Infantis, *Escherichia coli* ATCC 25922, *E. coli* O157:H7 ATCC 43890, *Acinetobacter*

Table 1.
Bacterial Inhibition Zone Diameters Formed by Water-Based and Ethanol-Based Propolis on Analyzed Bacteria

Morphology	Group	*n*	Mean ± SE (mm)	Median (Q1–Q3)	Minimum–Maximum	*p*
Enterococci	Ethanol	36	16.31 ± 0.52	16 (15–18)	6–23	<.001
	Water	36	8.83 ± 0.73	6 (6–14.25)	6–17	
Staphylococcus	Ethanol	36	15.03 ± 0.97	15 (10–19.25)	6–28	<.001
	Water	36	9.97 ± 0.6	10.5 (6–13)	6–16	
Bacillus spp.	Ethanol	6	19 ± 1.79	20 (16–21)	13–25	.005
	Water	6	11.5 ± 1.06	11 (10.25–13.25)	8–15	
Salmonella	Ethanol	18	12.5 ± 0.9	12 (9.25–15.75)	7–19	<.001
	Water	18	7.28 ± 0.59	6 (6–7)	6–14	
Escherichia coli	Ethanol	3	6 ± 0	6 (6–6)	6–6	NaN
	Water	3	6 ± 0	6 (6–6)	6–6	
Acinetobacter	Ethanol	3	22.67 ± 2.19	21 (20.5–24)	20–27	.008
	Water	3	8.67 ± 1.76	8 (7–10)	6–12	
Listeria	Ethanol	3	13.67 ± 3.93	16 (11–17.5)	6–19	.246
	Water	3	8.67 ± 2.67	14 (6–10)	6–14	
Pseudomonas	Ethanol	3	16.3 ± 0.88	16 (15.5–17)	15–18	.044
	Water	3	10 ± 2.3	10 (8–12)	6–14	
All	Ethanol	108	15.21 ± 0.48	15 (12–19)	6–28	<.001
	Water	108	9.05 ± 0.36	6 (6–12.3)	6–17	
Gram-positive	Ethanol	78	15.92 ± 0.53	16 (13.3–19)	6–28	<.001
	Water	78	9.56 ± 0.44	8 (6–13)	6–17	
Gram-negative	Ethanol	30	13.37 ± 0.98	13.5 (9–17.5)	6–27	<.001
	Water	30	7.7 ± 0.52	6 (6–8)	6–14	

Note: NaN = Not a number; *p* = Statistical significance of the effect of water- and ethanol-based propolis extracts on bacteria; SE = Standard error.

baumannii ATCC 19606, *Pseudomonas aeroginosa* ATCC 15442 cultures were used in the study as Gram-negative isolates. Thirty-six bacteria strains were incubated in 3 replicates and the total number of samples (*N*) was 108 on ethanol and water bases (Table 1). Bacterial cultures were used from the bacterial culture collection of Ankara University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology.

Determination of Bacterial Inhibition Zones

The antimicrobial activity of ethanol- and water-based propolis extracts against the specified foodborne pathogenic microorganisms was evaluated by determining the bacterial inhibition diameters (mm) using the agar-well diffusion method. The specified bacterial colonies were revived overnight at 37°C for 18 hours in brain heart infusion broth (Merck 110493). The ND-1000 nanodrop device was used to determine the turbidity of the overnight cultures and the turbidity of the isolates was measured at 600 nm wavelength; the values between 0.100 and 0.300 were accepted as 9 log cfu/mL and were used in the study (Bilir Ormanci et al., 2008). Colony counts were confirmed with the pour plate technique by inoculating on plate count agar (Oxoid CM0325). Afterward, they were inoculated on Mueller–Hinton agar (MHA, Merck 103872) using the swap technique. Then, four wells of equal diameter (6 mm) were opened on MHA agars in which the microorganisms were inoculated, and 60 µL of negative control (distilled water), positive control (ethanol 96%), ethanol-based propolis extract, and water-based propolis extract were placed in each well and incubated at 37°C for 48 hours. At the end of the incubation, the inhibition zone diameters (mm) were measured and the antimicrobial activity was evaluated. The experiments were independently repeated three times (Boyanova et al., 2005).

Statistical Analysis

Descriptive statistics were used to summarize the data and assumptions were tested with the Shapiro–Wilk test and *Q–Q* plot for normality and the Levene test for homogeneity of variances. Conveniently, independent sample *t*-tests or Mann–Whitney *U* tests were used to compare lengths of inhibitory zones for all 36 bacteria strains, Gram-positive and Gram-negative bacteria, along with eight different bacterial genera to compare ethanol- and water-based extracts' inhibitory effects. The quantized variables of diameters were expressed as mean ± standard error, median (first quartile Q1–third quartile Q3), minimum–maximum values, and significance was tested with $p < .05$ criterion. Statistical Package for the Social Sciences software package version 26.0 (IBM SPSS Corp., Armonk, NY, USA) was used for statistical analysis.

Results

Considerable and strong growth inhibition zone diameters were adapted from Boyanova et al. (2005). Both ethanol- and water-based propolis extracts had an inhibitory effect against all isolates besides *E. coli*, and ethanol-based propolis extract was highly effective against 66.6% (24/36) of the isolates. It was observed that the propolis tested in the study showed the most inhibitory effect on *Acinetobacter baumannii* (Figure 1). While none of the isolates were inhibited by water-based propolis, 80.7% (21/26) of

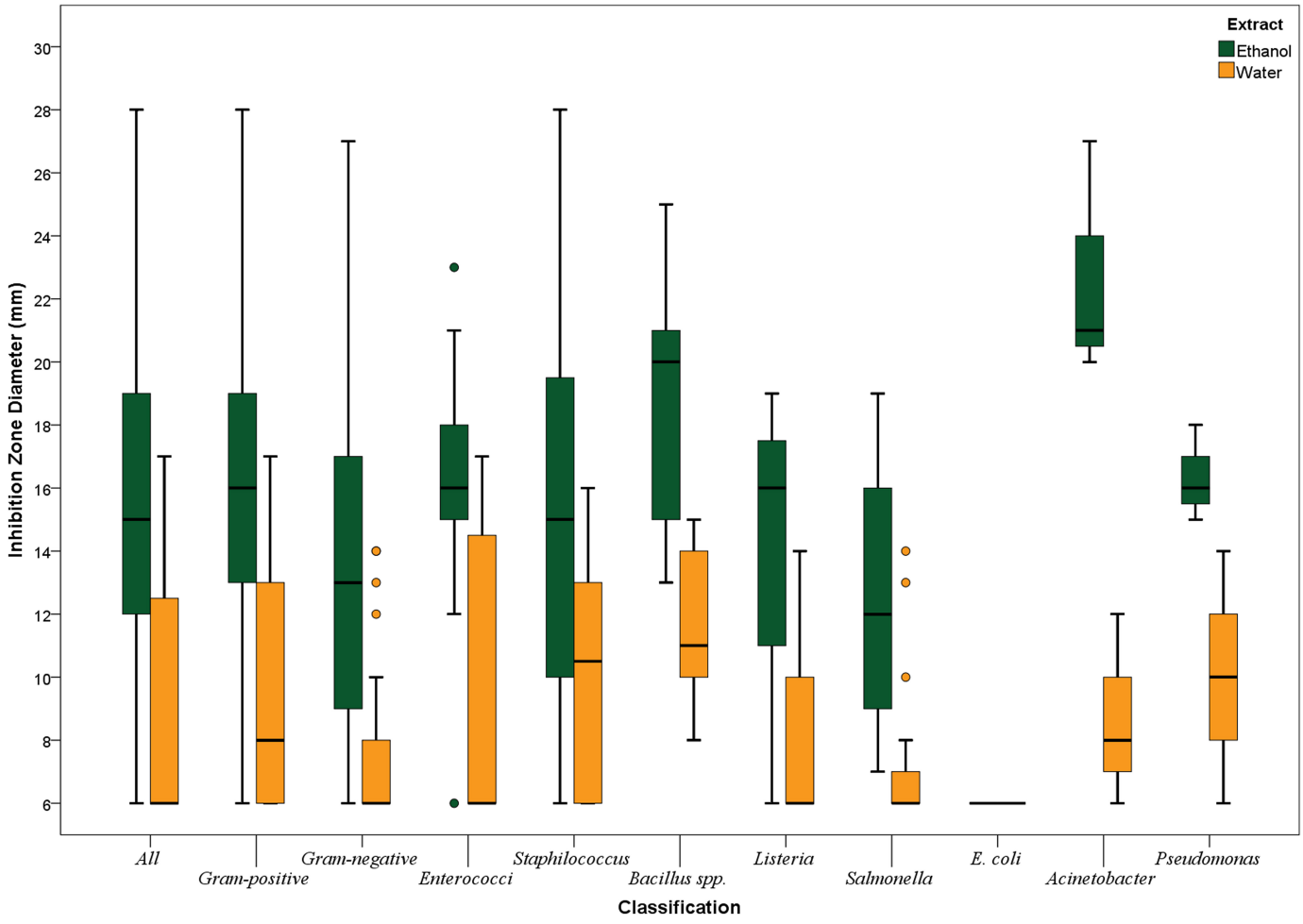

Figure 1.
Distribution of Bacterial Inhibition Zone Diameters (mm) Grouped by Extraction Type on Bacteria.

Gram-positive isolates and 30% (3/10) of Gram-negative isolates were determined to be inhibited by ethanol-based propolis (≥15 was considered as significant growth inhibition zone). On the other hand, 23% of the Gram-positive isolates (6/26) and 10% of the Gram-negative isolates (1/10) were determined to be strongly inhibited (≥20 as the strong growth inhibition zone) by ethanol-based propolis. Growth inhibition zones of water (W) and ethanol (E)-based propolis on *Staphylococcus aureus* SEB+ and *Bacillus subtilis* subsp. *spizizenii* are given in Figure 2.

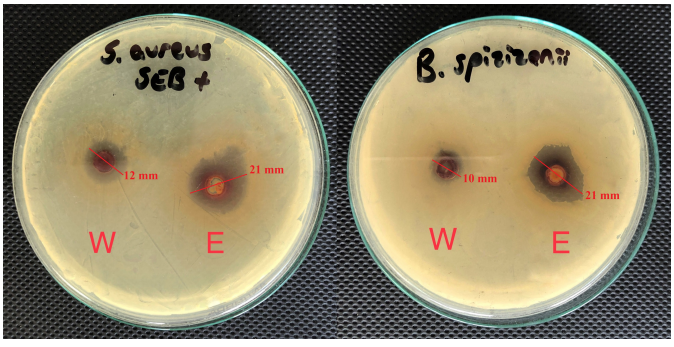

Figure 2.
Bacterial Inhibition Zones Formed by Water- and Ethanol-Based Propolis on Staphylococcus aureus SEB+ and Bacillus subtilis subsp. *spizizenii. E = Ethanol-based ethanol extract; W = Water-based ethanol extract;.*

The study separated 36 bacterial strains based on their genus and conducted statistical analysis to compare the bacterial inhibition zone diameters formed by water-based and ethanol-based propolis. The results are presented in Table 1. In addition, we used box plots to display the size distributions of the bacterial inhibition zones (measured in mm) formed by water and ethanol-based propolis (Figure 1).

As a result of the statistical analysis, it was determined that only ethanol-based propolis of Turkey was effective against enterococci, *S. aureus*, *B. cereus*, *B. spizizenii*, *Salmonella*, *L. monocytogenes, A. baumannii,* and *P. aeroginosa*, except *E. coli*. In *E. coli*, all inhibition diameter measurements were 6 mm; since there was no distribution in the data, statistical analysis could not be performed and therefore was expressed as Not A Number (NaN) in Table 1. In addition, ethanol-based propolis extracts were more effective in inhibiting bacteria than water-based extracts (Figure 1). When based on genera examinations determined, there was a significant difference between the effectiveness of ethanol- and water-based extracts on all genera of isolates except *L. monocytogenes* (Table 1). On the other hand, ethanol-based extract was found to have a significant inhibitory effect against 91.6% (11/12) of enterococci, 63.6% (7/11) of *S. aureus*, all of the *Bacillus* (2/2), *Listeria* (1/1), *Acinetobacter* (1/1), *Pseudomonas* (1/1), and 16.6% (1/6) of *Salmonella* isolates. However, it was determined that it did not have any inhibitory effect against *E. coli* isolates.

Discussion

Despite the broad knowledge in this area, only a small number of earlier studies have reported propolis' antimicrobial effects on various foodborne pathogen bacteria. In general, studies on this subject have been limited to only a few microorganisms (Afata et al., 2022; Hegazi et al., 2000; Hünler Dönmez, 2021; Postali et al., 2022; Touzani et al., 2021). Our study was carried out using 36 bacterial strains belonging to 8 different bacterial genera. On the other hand, it is noteworthy that in studies examining the antimicrobial effect of propolis extracts, only ethanol-based extracts have been generally used, while studies on water-based extracts have been lacking. Recently, propolis preparations have been offered for sale as water-based as well as ethanol-based extracts. The sale and consumption of water-based propolis extracts have been becoming more common day by day due to religious prohibitions, the thought that it is more suitable for use by babies, and organic. The crucial point is that, despite the fact that consumers prefer water-based extracts, they believe these products will provide them with the same level of effectiveness as ethanol-based extracts. Investigating whether there is a difference in the antibacterial activity of ethanol-based and water-based propolis extracts is essential for this reason. Therefore, in our study, the efficacy of propolis extracts in ethanol and water, the products both from the same brand and available as ready-to-use in Turkey, were examined. Similar to the studies of Bakkaloğlu et al. (2021) and Hünler Dönmez (2021), while water-based extracts were not found to be effective against any bacteria in our study, it was reported in a recent study that extractions of water-based extracts at different temperatures were effective against different pathogens. The study examining the antimicrobial effect of water-based propolis extracts on foodborne pathogens by Ömer et al. (2023) reported that the extraction temperature of propolis has a strong effect on the antimicrobial effect, and the antimicrobial activity of the extract increases as the extraction temperature increases.

Determination of bacterial inhibition zones using the agar-well diffusion method and obtaining information about the antimicrobial resistance of microorganisms is an accepted approach reported by researchers (Boyanova et al., 2005; Postali et al., 2022). In other studies, unlike our study, in addition to the agar-well diffusion method, the broth dilution method is also used to determine the minimum inhibition concentration (MIC) of the extracts against the isolates (Gummuluri et al., 2019; Stepanović et al., 2003). In the study by Ömer et al. (2023), one of the few studies examining the antimicrobial effect of water-based propolis extracts on foodborne pathogens, the results were reported using only the MIC method. Because we aimed to evaluate the antibacterial activity of propolis extracts available in the market and the propolis was not extracted by us, the MIC was not investigated in this study. Our work is unique in terms of both comparing the antimicrobial effect of ethanol- and water-based propolis extracts and determining the effectiveness with an easier method such as the agar-well method.

In this study, the inhibition zone formed by ethanol- and water-based propolis against 26 Gram-positive and 10 Gram-negative foodborne bacteria was determined by determining the bacterial inhibition zone diameters. Statistical analysis results showed that ethanol-based propolis was found to be more effective than water-based propolis, and only ethanol-based extract had an inhibitory effect against bacteria belonging to enterococci, *S. aureus*, *Bacillus*, *Listeria*, *Acinetobacter*, *Pseudomonas*, and *Salmonella* genus, but did not create a significant growth inhibition zone against *E. coli* isolates. Therefore, we can conclude that ethanol-based propolis extract was more effective against Gram-positive bacteria than against Gram-negative bacteria, as in other similar studies (Boyanova et al., 2005; Stepanović et al., 2003). On the contrary, in a recent study examining the antimicrobial activity of Ethiopian propolis, it was determined that propolis collected from two different regions did not show antimicrobial activity against *S. aureus*, and it was stated that propolis samples were more active against Gram-negative bacteria than Gram-positive. Since only *E. coli* and *S. aureus* were used in the aforementioned study and statistical analysis may not have been performed, such a conclusion may have been drawn. In this study, it was also determined that propolis samples from the Middle East region showed high activity for both *S. aureus* and *E. coli* strains (Afata et al., 2022). On the other hand, the lowest activity was shown in propolis samples from Germany, Ireland, and Korea (Al-Ani et al., 2018; Kim et al., 2011). On the other hand, Brazilian red propolis is reported to be effective against both *Salmonella* and *S. aureus* (Dos Santos et al., 2021). As stated in the study of Bakkaloğlu et al. (2021), the antifungal activity of Turkish propolis is stronger than the antibacterial activity of propolis extracts.

The ethanol-based extract had an inhibitory effect only on *S.* Pullorum NCTC 10705 isolate, out of six *Salmonella* species used in our study. Although considerable antimicrobial activity was observed in only this *Salmonella* isolate, the difference between the effectiveness of ethanol- and water-based extracts on *Salmonella* was statistically significant when the *Salmonella* isolates were examined on a genus basis. In a study examining the antimicrobial activity of propolis on *Salmonella*, it was determined that Greek propolis had no antimicrobial effect on *S. typhimurium* (Postali et al., 2022). On the other hand, in a study examining the antimicrobial activity of Brazilian red propolis on *S. enteritidis*, unlike our study, the inhibition zone was found to be 15 and above (in ours = 12, Table 1) and it was interpreted that propolis showed antimicrobial resistance on this bacterium (dos Santos et al., 2021). This difference can be explained by the fact that the activity of the phytochemicals extracted from propolis was examined, not the propolis itself, in the mentioned study.

The results showed that both ethanol- and water-based propolis extracts were not effective against both *E. coli* ATCC 25922 and *E. coli* O157 ATCC 43890 strains. It means, *E. coli* was proven to be the most resistant of all the tested bacteria. On the other hand, at least ethanol-based propolis extract was found to be effective against all 35 bacterial cultures except *E.coli*. In a study in which the antimicrobial activities of Austrian, German, and French propolis were determined, it was stated that all three propolis samples were effective against *E. coli* and *S. aureus* isolates, but German propolis showed the highest antibacterial activity against these bacteria, and French propolis was less effective (Hegazi et al., 2000). In several other studies, it was also reported that propolis samples from Turkey, Oman, and Slovakia showed antimicrobial activity against *E. coli* isolates (Afata et al., 2022; Mavri et al., 2012; Popova et al., 2013; Uzel et al., 2005). The reason why propolis samples were found to be ineffective against *E. coli* cultures in our study may be that only ATCC cultures of *E. coli* were used in our study.

In our study, although ethanol-based extract was seen to be more effective, statistically no difference was found between the

antimicrobial effect of ethanol- and water-based propolis against *L. monocytogenes*. It is thought that this may be due to the fact that only one *Listeria* isolate was studied three times in our study. In a study examining susceptibility to propolis extracts on *L. monocytogenes*, different wild *Listeria* strains were indicated as susceptible to all propolis extracts. The antibacterial effect of propolis on *Listeria* species showed that all propolis inhibited this foodborne pathogen (Rendueles et al., 2023). On the other hand, Postali et al. (2022) stated that propolis extracts were effective on *L. monocytogenes*, but it is noteworthy that the inhibition zones they stated were much lower than ours.

It was observed that the propolis tested in the study showed the most inhibitory effect on *Acinetobacter baumannii*. *Acinetobacter baumannii* is known as a significant public health problem today. In one of the few studies investigating the effect of propolis on this pathogen, the inhibitory effect of Sargodha and Lahore propolis on *A. baumannii* was investigated, and it was observed that Sargodha propolis was similar to the inhibition zone diameters obtained in our study, but Lahore propolis had a lower inhibitory effect than the inhibition zones obtained in our study (Hannan et al., 2015).

It has been reported that bee species, geographical origin, climate and storage conditions, extract preparation, and, as a result of all these, different phenolic compounds in the content of propolis were effective on the antibacterial activity of propolis (Garzoli et al., 2023; Touzani et al., 2021). For example, it has been stated that propolis of Swiss origin was rich in phenolic glycerides and those from Sicily were rich in diterpenic acids (Bankova et al., 2002). On the other hand, propolis from Algeria, Bulgaria, Greece, and Turkey is generally reported to contain predominantly flavonoids and esters of caffeic and ferulic acids (Bakkaloğlu et al., 2021; Velikova et al., 2000).

Conclusion and Recommendations

As a result, commercial ethanol-based propolis tested in this study had an antimicrobial effect, especially on Gram-positive foodborne pathogens. It was also determined that the effectiveness of water-based propolis on the studied foodborne pathogens was statistically low compared to ethanol-based propolis extracts. There are different extraction methods and optimization studies for water-based propolis products. We think that more studies are needed to reveal the efficacy of water-based propolis, which is widely sold and consumed worldwide, especially the differences in the contents of commercial water- and ethanol-based propolis extracts. The determination of these components in new studies that can be done will provide more detailed information about the antimicrobial effect of propolis extracts.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – B.O.A.; Design – B.O.A., E.G.; Supervision – B.O.A.; Resources – B.O.A.; Materials – B.O.A.; Data Collection and/or Processing – B.O.A., E.G.; Analysis and/or Interpretation – B.O.A., E.G.; Literature Search – B.O.A.; Writing Manuscript – B.O.A., E.G.; Critical Review – B.O.A., E.G.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – B.O.A.; Tasarım – B.O.A., E.G.; Denetleme – B.O.A.; Kaynaklar – B.O.A.; Malzemeler – B.O.A.; Veri Toplanması ve/veya İşlemesi – B.O.A., E.G.; Analiz ve/veya Yorum – B.O.A., E.G.; Literatür Taraması – B.O.A.; Yazıyı Yazan – B.O.A., E.G.; Eleştirel İnceleme – B.O.A., E.G.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

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