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Original Research

Determination of the Antimicrobial Activity of Four Different Tea Extracts Against Foodborne Pathogens

Emine Dinçer[1], Nurcan Bağlam[2]

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Abstract

Objectives: Due to the presence of various bioactive compounds including polyphenols and saponins, tea exhibits antimicrobial activity against microorganisms. The aim of this study is to investigate the antimicrobial activity of black, white, green, and oolong tea against some foodborne pathogens.
Materials and Methods: For the extraction of tea samples, the Soxhlet method was chosen, and MIC values were determined using the broth micro-dilution method for the evaluation of antimicrobial activity. The selection of tea varieties used in the study was determined based on their consumption frequencies and popularity. *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 7829, *Staphylococcus aureus* ATCC 292123, and 3 *Listeria monocytogenes* (*L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 7644, and *L. monocytogenes* 4b ATCC 19115) strains were used as indicator microorganisms in the study.
Results: The highest antimicrobial activity was observed against *L. monocytogenes* strains in white tea, with an MIC value of 0.256 mg/mL. The comparison of antimicrobial activity of different tea types revealed that black tea exhibited lower antimicrobial activity compared to other tea varieties.
Conclusion: The findings of the current study indicate that non-fermented tea types, such as white and green tea, which are known to be more effective in terms of healthy nutrition, exhibit higher antimicrobial activity. Therefore, it is believed that non-fermented tea varieties would be more effective in combating pathogenic microorganisms.

Keywords: *Antimicrobial activities, pathogenic microorganisms, tea extracts*

[1]**Emine Dinçer (Corresponding Author).** Sivas Cumhuriyet University, Faculty of Health Science, Department of Nutrition and Dietetics, Sivas, Turkiye. Phone Number: 0346 487 0000, e-mail: edincer@cumhuriyet.edu.tr
[2]**Nurcan Bağlam.** Sivas Cumhuriyet University, Faculty of Health Science, Department of Nutrition and Dietetics, Sivas, Turkiye. Phone Number: 0346 487 0000, e-mail: nurcanbaglam@cumhuriyet.edu.tr

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Introduction

Tea, a beverage made from the leaves of *Camellia sinensis*, originated in China and has become increasingly popular worldwide in recent years (Tang et al, 2019b). More than 3 billion people in over 160 countries and regions worldwide consume tea. The tea plant is classified into six categories based on the processing of its leaves: white, yellow, green, oolong, black, and dark tea (Hinojosa et al., 2021; Pan et al., 2022). Among them, mostly black (fermented), green (unfermented), oolong (semi-fermented), and white (unfermented, using only immature leaf buds) teas are available in the markets. However, a few other types, such as yellow, pu-erh, etc. teas, are sold according to the local demand (Hazra et al., 2019; Halder et al., 2020). Indeed, tea, which is considered the most widely consumed beverage in the world after water, has been the subject of extensive research in recent years due to its chemical composition (Khan & Mukhtar, 2019). Turkey ranks among the leading countries in the world in terms of tea consumption. In line with literature data, the most commonly consumed type of tea in Turkey is black tea, followed by green tea, yellow tea, and oolong tea, especially in the western regions (Yildirim & Karaca, 2022).

Tea contains various bioactive compounds such as polyphenols, pigments, polysaccharides, alkaloids, free amino acids, and saponins (Bi et al., 2016; Tang et al., 2019b; Zhao et al., 2019). Many studies have shown that tea and its bioactive components, such as polyphenols, pigments, polysaccharides, alkaloids, free amino acids, and saponins, have health benefits including antioxidant, anti-inflammatory, antibacterial, immune-regulating, anti-cancer, cardiovascular health promotion, diabetes prevention, obesity prevention, and liver protection (Tang et al., 2019a). It is reported that there are around 4000 bioactive compounds in tea leaves, and one-third of them are polyphenols (Das et al., 2022). Indeed, tea contains polyphenols such as flavonols and flavones, but in much smaller quantities compared to catechins and other major compounds (Wong et al., 2022). It is known that catechins play a key role in most of the biological activities of tea (Liu et al, 2022). Thus, all types of tea have varying amounts of catechins depending on their degree of oxidation. The presence of different forms and derivatives of catechins in different types of tea has allowed these compounds to possess different biological activities. The catechins belonging to the flavonoid group found in tea are (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epicatechin (EC). Unfermented green tea is considered the best source of catechins (Zhang et al., 2019). Due to the complete fermentation process that black tea leaves undergo and the conversion of catechins into theaflavins, the catechin content in black tea is lower compared to green tea (Musial et al., 2020). Oolong tea undergoes a processing method with a shorter oxidation period, resulting in the presence of both catechins and theaflavins in its content. Unlike the complex processes involved in other types of tea, white tea is primarily obtained through plucking, withering, and drying processes (Zhou et al, 2023). Researchers also indicate that white tea has a higher polyphenol content and antioxidant activity compared to other types of tea (Purwaningtyas & Shobib, 2022). Having the highest amino acid content is also one of the important characteristics of white tea (Wong et al., 2022). Different processing methods give a distinctive taste, aroma, and color to tea but also affect the ratio of active ingredients and thus their biological activities (Liu et al, 2022).

With increasing consumption, natural polyphenols such as tea polyphenols are considered a safer option for consumption due to their antibacterial potential when compared to chemicals or artificially prepared food additives (Zhang et al., 2021). There are numerous studies claiming that some of the bioactive compounds in tea, have beneficial effects such as antimicrobial activity (Nibir et al., 2017; Shah et al., 2018; Kiyama, 2020; Yan et al., 2020). According to current literature, many studies suggest that polyphenols obtained from tea extracts, particularly EGCG and ECG, have beneficial effects against foodborne pathogenic microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, *Clostridium botulinum*, *Bacillus cereus, Salmonella enterica, L. monocytogenes*, and *S. aureus* (Kiyama, 2020; Liao et al., 2020; Yan et al., 2020). These types of pathogenic microorganisms, which cause serious economic losses in the food industry, contaminate food and cause toxin production that seriously affects human health (Imre et al., 2020). Diseases caused by pathogens such as *S. aureus* and *E. coli* are frequently seen both in our country and in other countries. *L. monocytogenes* is gram-positive, rod-shaped, facultative anaerobic bacteria that can cause infections in high-risk groups, especially the elderly, pregnant women, and newborns. Although not as common as diseases caused by other foodborne pathogens such as *Salmonella*, *Campylobacter*, or verotoxigenic *E. coli*, listeriosis is important due to its high mortality rate (Charlier et al., 2017). The latest report published by the European Food Safety Authority (EFSA) in 2018 indicates that *L. monocytogenes* ranks high among foodborne outbreaks, and the mortality rate was 13.8% in 2480 cases of listeriosis (EFSA & ECDC, 2018).

Although it is known that the tea plant exhibits antimicrobial activity due to its bioactive compounds, there is still limited information available regarding the antimicrobial effects of different tea varieties on various types of microorganisms. In this context, there is a need for new studies that specifically investigate the antimicrobial effects of different teas on different microorganisms and compare different tea types. The aim of the current study was to investigate

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the antimicrobial activities of white tea, green tea, oolong tea, and black tea extracts against foodborne pathogens.

Material and Methods

Material

To evaluate the antimicrobial activity of different tea samples, black, white, green, and oolong tea samples in sealed packages were randomly purchased from the commercial markets in Sivas province. Muller Hilton Broth (MHB) and absolute ethyl alcohol (%99.8) were obtained from Merck, Turkey. *E. coli* ATCC 25922, *P. vulgaris* ATCC 7829, *S. aureus* ATCC 292123, and 3 *L. monocytogenes* (*L. monocytogenes* ATCC 19111, *L. monocytogenes* ATCC 7644, *L. monocytogenes* 4b ATCC 19115) was chosen as indicator foodborne pathogen microorganisms. All microorganisms were purchased from the American Type Culture Collection, Manassas, Virginia, USA. They were stored at -80°C in in 20% glycerol (v/v) and were pre-cultivated twice in Muller Hilton Broth at 37 °C for 18 h, before they were used.

Extraction Procedure

Extraction from tea samples was performed using the soxhlet extraction method (Harborne, 1984; De Castro & Priego-Capote, 2010). For the extraction, 50 g each tea samples (commercially available dried tea) were separately filled in f sets of soxhlet apparatus, 400 mL absolute ethyl alcohol was used as solvent, and extraction process was carried out for 12 hours. At the end of the extraction procedure, the solvent was removed at 40°C with the evaporator, and a dry-pure substance was obtained. The dry-pure substance was stored at +4°C. For the main stock preparation, after 2 g dry-pure substance was dissolved in 5 mL of ethanol, filtered through Whatman paper and was used for analyzes on the same day.

Antimicrobial Activity Assay

The antimicrobial activity of the tea extracts against the foodborne pathogen microorganisms was determined using the broth micro-dilution method. Clinical Laboratory Standard Institute (CLSI) guidelines were followed for the assay (CLSI, 2014).

To analyze the samples, two-fold serial dilutions of tea extracts were prepared from the main stock, varying in concentration from 4.096 mg/mL to 0.64 mg/mL in MHB. Each dilution of tea extracts (100 µL) was transferred to sterile 96 well microtitration plate. Then, the optical densities of overnight second active cultures of indicator microorganism in MHB were adjusted to McFarland standard No: 0.5 using the same medium and 100 µL of this solution was added each well. Plates were incubated at 37 °C for 24 h. At the end of the incubation period, the absorbance was recorded at 600 nm by BMG LABTECH's ultra-fast UV/vis spectrophotometer

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(SPECTROstar Nano, Germany). The lowest concentration that inhibits the indicator microorganism growth was determined as the minimum inhibitory concentration (MIC) value. Ciprofloxacin was preferred as the reference antimicrobial agent and used as the positive control. Density-adjusted indicator microorganism solution (200 µL) was used as negative control. In addition to determining the MIC values, to evaluate the antibactericidal effect of sub-MIC concentrations, the inhibition ratio (%) was calculated using the ‘‘$100 – [(OD_{T2}-OD_{T1}) / (OD_{NC}-OD_{PC})] *100$’’ formula (2); where the OD_{T2} is the absorbance of the bacterial solutions after incubating growth medium containing dilutions of the tea extracts, OD_{T1} is the absorbance of medium containing only dilutions of the tea extracts, OD_{NC} is the absorbance of the negative control and OD_{PC} is the absorbance of the positive control.

Statistical Analysis

All assay was performed in triplicate and the mean ± standard deviation of repetitions was used for the inhibition ratio calculations. The data obtained from the study were analyzed using the SPSS 23.0 program. Comparisons between the groups were analyzed using the Kruskal-Wallis test. p values less than 0.05 were considered statistically significant.

Result

It was determined that the highest antimicrobial activity was exhibited by white tea extract against *L. monocytogenes* ATCC 19111 and *L. monocytogenes* 4b ATCC 19115 with a MIC value of 0.256 mg/mL. MIC values were given in Table 1 in detail.

Table 1: Minimum Inhibitory Concentrations (MIC, mg/mL) of Different Tea Extracts Against Foodborne Pathogen Microorganisms

	Black Tea	White Tea	Green Tea	Oolong
E. coli ATCC 25922	> 4.096	> 4.096	4.096	4.096
S. aureus ATCC 292123	2.048	1.024	0.512	0.512
P. vulgaris ATCC 7829	4.096	0.512	0.512	0.512
L. monocytogenes ATCC 7644	1.024	1.024	2.048	1.024
L. monocytogenes ATCC 19111	> 4.096	0.256	4.096	2.048
L. monocytogenes 4b ATCC 19115	> 4.096	0.256	4.096	1.024

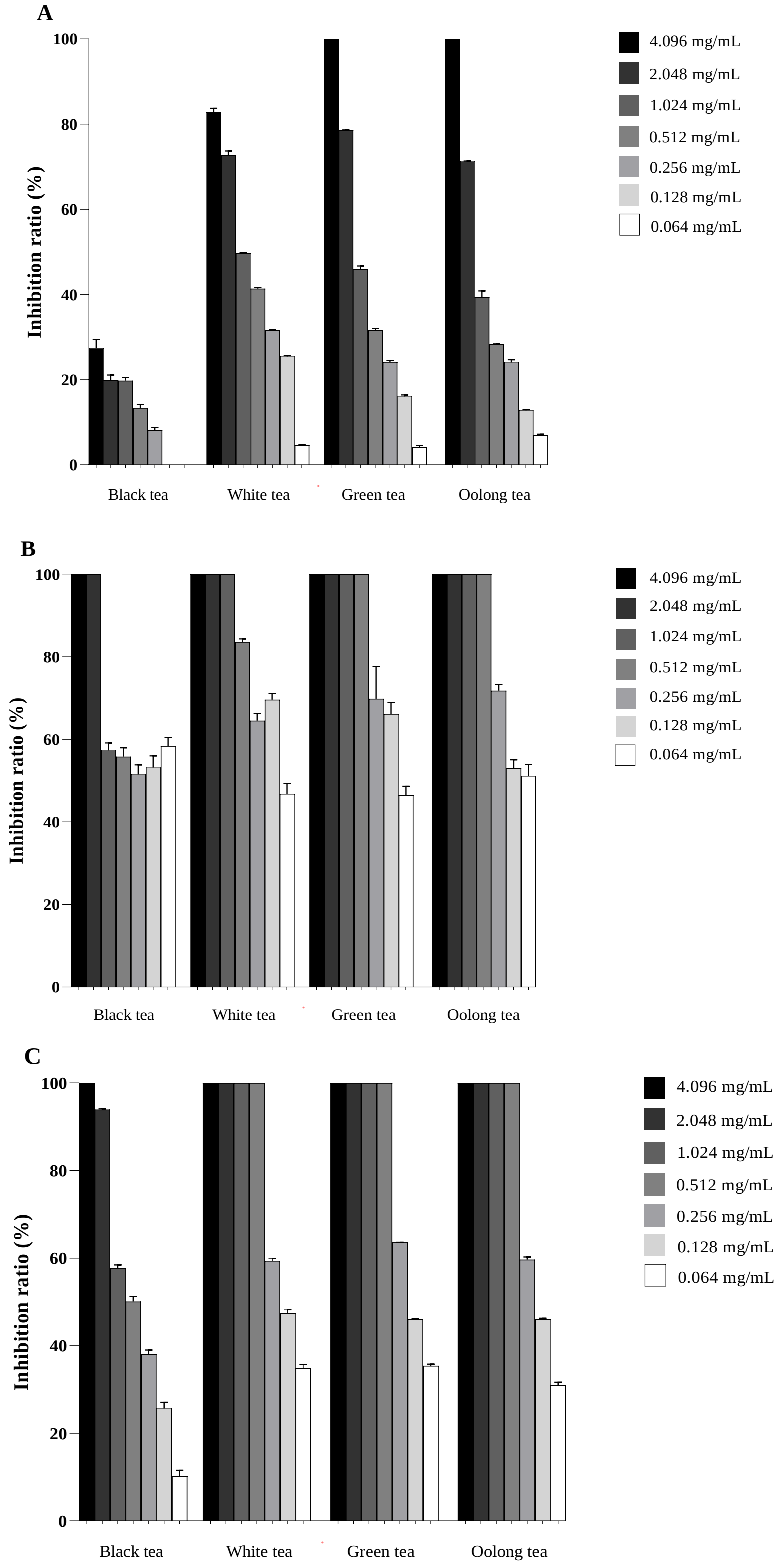

Figure 1: Inhibitory Effect of Different Tea Extracts in Various Concentrations on *E. coli*, *S. aureus*, *P. vulgaris*

*A: *E.coli* ATCC 25922, B: *S. aureus* ATCC 292123, C: *P. vulgaris* ATCC 7829
**Statistical significance between the tea extracts $p>0.05$.

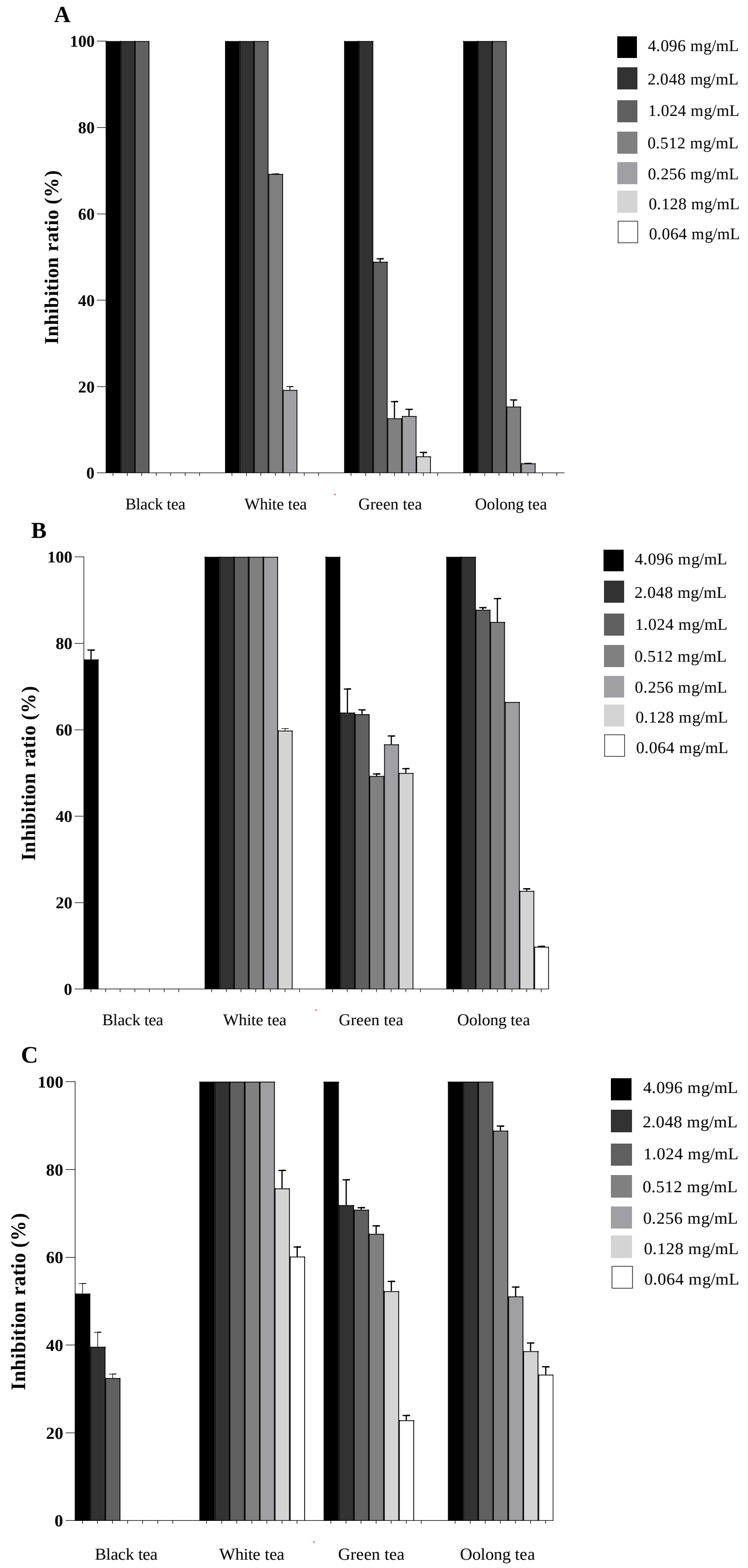

Figure 2: Inhibitory Effect of Different Tea Extracts in Various Concentrations on *Listeria species*

*A: *L. monocytogenes* ATCC 7644, B: *L. monocytogenes* ATCC 19111, C: *L. monocytogenes* 4b ATCC 19115
**Statistical significance between the tea extracts $p>0.05$

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The results show that all of the tea extracts exhibited the lowest antimicrobial activity against *E. coli*. A general evaluation indicates that the antimicrobial activity of black tea extracts is weaker compared to other types of tea. In the study, besides the MIC values of tea extracts, varying in concentration from 4.096 mg/mL to 0.64 mg/mL inhibition rate (%) was calculated. The evaluations show that all types of tea, especially white and green tea, are effective even at Sub-MIC concentrations (Fig. 1 and Fig. 2). There was no statistical significance between the sub-mic values of tea extracts ($p>0.05$).

Discussion

Within the context of the present study, the antimicrobial effect of extracts obtained from tea types frequently consumed in our country has been evaluated on certain foodborne pathogens. Foodborne diseases cause serious health issues, deaths, and economic losses (Gourama, 2020). The Center for Disease Control and Prevention (CDC) reports that approximately 48 million people in the United States are infected with foodborne pathogens each year. For 2018, a total of 5,146 foodborne and waterborne outbreaks, were reported in Europe. In these outbreaks, 48,365 cases were reported, and 40 deaths were recorded (EFSA & ECDC, 2019). It should also be noted that there may be unrecorded or unreported cases. Certain segments of the population, especially infants, pregnant women, the elderly, immunocompromised individuals, the homeless, and those with low socioeconomic status, are more vulnerable to foodborne illnesses. The pathogenesis of foodborne illnesses is quite diverse; pathogens such as bacteria and viruses, parasites, and chemicals can cause the disease through contaminated foods (Foddai & Grant, 2020). These pathogens can be transmitted through various sources, such as animal and plant-based foods, soil, water, air, equipment, and humans. These microorganisms can contaminate a wide variety of foods, such as meat, dairy products, fruits, vegetables, seafood, grains, and water (Gourama, 2020).

Recent epidemiological studies have linked tea consumption to a variety of health advantages (Yi et al., 2019). The antimicrobial property of tea is mainly associated with the polyphenols present in its structure. The main polyphenols found in tea are flavonoids. The most commonly found types of flavonoids in tea are flavanols (catechins) and flavonols (quercetin, kaempferol, and myricetin). Especially catechins among these, as antioxidant polyphenols, inhibit the growth of bacteria. The dominant catechins among these are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) (Kumar et al., 2012). Studies have shown

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that different types of tea have varying degrees of antibacterial activity against pathogenic microorganisms. In a study by Kumar et al. (2012) investigating the antibacterial effect of green tea extract on six environmental pathogens, including *S. aureus, Streptococcus, Pseudomonas, E. coli, Bacillus, and Proteus*, it was reported that green tea had strong antibacterial activity against these bacteria. Furthermore, Kadiroğlu et al. (2017) also suggest that black tea and green tea extracts have antimicrobial activity against *S. aureus* and *Pseudomonas aeruginosa* but not *E. coli*. Similarly, in the present study, it was observed that tea extracts exhibited varying degrees of activity against *E. coli, S. aureus, P. vulgaris*, and *L. monocytogenes*. Similarly, in the present study, it was observed that tea extracts exhibited varying degrees of activity against *E. coli*, *S. aureus*, *P. vulgaris*, and *L. monocytogenes*. In the current literature, it is observed that different researchers report varying results regarding the antimicrobial activity of tea varieties. These differences can be attributed to factors such as the use of different extraction methods, the selection of different indicator microorganisms, and variations in extract concentrations across studies.

In the present study, it was observed that oolong and green tea were more effective against *E. coli* than black and white tea. Furthermore, it was found that the MIC value of black and white tea for *E. coli* was not within the studied concentration range but rather above 4.096 mg/mL. Similarly, Fahmi et al. (2022) evaluated the inhibitory effect of ethanol extracts obtained from green tea leaves (*C. sinensis*) against E. coli and reported that the antibacterial effect of green tea ethanol extract increased as the concentration increased and that green tea could be used as an antibacterial agent against *E. coli*, but the effectiveness of black tea was lower. Another study conducted by Zihadi et al. (2019) reported that the MIC value of green tea extract for *E. coli was* 31.25 mg/mL. According to the evaluation of the antimicrobial activity of the tea extracts used in the current study against *S. aureus*, it was found that green and oolong teas showed stronger antimicrobial activity compared to black and white teas. In their study, Zihadi et al. (2019) investigated the antibacterial potential of green tea extract on methicillin-resistant *S. aureus* (MRSA) and found that it was effective against MRSA with a minimum inhibitory concentration (MIC) value of 31.25 mg/mL. Current literature suggests that tea samples are expected to have an effect on *E. coli* and *S. aureus*. It is known that EGCG, one of the dominant flavonoids in tea composition, exhibits its effectiveness by damaging the liposome membrane of *E. coli* and *S. aureus* (Yang & Zhang, 2019).

Listeriosis has a low prevalence, but because of its high fatality rate and potential for complications, it is nonetheless regarded as a significant public health issue (EFSA & ECDC, 2019). In the present study, the antimicrobial activity of tea extracts was evaluated against three

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different types of *L. monocytogenes* cultures, including one of serotype 4b. It was found that white tea, especially compared to other types of tea, exhibited a strong antimicrobial effect against *L. monocytogenes* ATCC 19111 and *L. monocytogenes* 4b ATCC 19115. Similarly, Mbata et al. (2008) reported that methanol and water extracts of green tea were effective against *L. monocytogenes*. In a study by Granato et al. (2016) evaluating the antimicrobial activity of black, green, and white tea extracts against some foodborne pathogens, similar to our study, it was reported that white tea exhibited the best efficacy against *L. monocytogenes*. In another study, Akbulut et al. (2020) compared the antimicrobial activity of black, green, and white tea against *L. monocytogenes* and reported that white and green tea were effective even at low concentrations. Camargo et al. (2016) investigated the antioxidant and antimicrobial properties of black, green, and white tea and found that white tea had the highest total phenolic content. They explained this by the oxidation of catechins to theaflavins and thearubigins during the fermentation stage of black tea production.

When the data obtained from the studies comparing the antimicrobial activity of different tea types is evaluated together, it is observed that black tea has the weakest antimicrobial activity. In the present study, it was found that especially white and green teas showed high antimicrobial activity. It is believed that this is due to the fact that white and green tea are produced without fermentation. It is well known that the components present in the tea plant, such as polyphenols, beta-carotene, lycopene, and tannins, possess strong antimicrobial potential. In particular, catechins, which are classified as a subgroup of flavonoids, have been shown to contribute to the antimicrobial properties of tea. On the other hand, the processing methods of teas significantly influence their polyphenol content and types. Black tea is produced through a process where the leaves undergo complete oxidation. This process increases the oxidation of polyphenols in tea and leads to the transformation of certain compounds. As a result, black tea is known to contain a lower amount of polyphenols and catechins compared to other tea types. Green tea, on the other hand, is produced through a process where the leaves undergo slight oxidation and are heat-treated. This process ensures the preservation of polyphenols and antioxidants in green tea to a significant extent. White tea, which does not undergo any fermentation process, is produced from tea leaves consisting of one bud and two leaves. In buds and young leaves, the characteristic substances that impact tea quality, such as phenolic compounds and caffeine levels, are more prevalent Therefore, white tea contains a high amount of antioxidant compounds, which explains its higher antimicrobial activity (Nibir et al., 2017; Shah et al., 2018; Feng et al., 2022). However, it should be noted that the current study has certain limitations, including the lack of determination of total

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phenolic content in the tea extracts and the absence of identification of specific bioactive compounds present in the extracts. Within the scope of this study, these constraints make it difficult to reach a firm judgment about the source of the observed antibacterial activity. Another limitation of the study is the lack of investigation into the impact of various factors, such as the production processes of commercially available tea types and the cultivation conditions of tea plants, on the antimicrobial activity. Additionally, another important limitation of the study is the use of a single extraction method, without comparing the antimicrobial activities of extracts obtained using different methods and solvents.

Conclusion

Foodborne microbial pathogens that contaminate food have long been a concern for humans. Chemical agents are the most common way to reduce the harm caused by foodborne pathogens. However, frequent and inappropriate use of these agents can increase antibiotic resistance in pathogenic bacteria and cause negative effects on the human body. In recent years, consumption of green tea, oolong tea, and white tea in both hot and cold forms has increased as part of popular diets. Tea has a preventive potential against many diseases and foodborne pathogens due to its flavanol and flavanol type polyphenols. In the current study, which determined the antimicrobial activity of 4 different tea varieties on 6 different foodborne pathogens, it was determined that white tea was effective on Listeria species even at low concentrations. More studies are needed to explain the possible mechanisms underlying these health effects and the active components, as well as to determine their effects on human health and define appropriate doses. Therefore, it is hoped that the current study will shed light on future research in this field. In the next stages of this study, it is crucial to focus on the isolation and identification of antimicrobial compounds to shed light on their mechanism of action. This step is of great significance in order to further understand the antimicrobial activity observed and unravel the underlying mechanisms.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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