Uludag Bee Journal
Uludag Bee J.
e-ISSN: 2687-5594
ISSN 1303-0248
https://dergipark.org.tr/en/pub/uluaricilik
https://uadubj.uludag.edu.tr/

Comparative in vitro antibacterial activities of chestnut and flower honeys against selected pathogenic bacteria

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Received: 15.04.2026 Accepted: 24.05.2026 Published: 01.07.2026

Abstract

The antibacterial properties of natural products have attracted increasing attention in recent years amid rising antimicrobial resistance. The present study evaluated the antibacterial activity of chestnut and flower honeys, sourced from various provinces of Türkiye. The honeys were tested at 100% and 25% concentrations. The standard strains utilised in this study encompassed *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 43894), Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300), and *Salmonella enterica* serovar *Enteritidis* (ATCC 13076). The antibacterial activity was determined using the agar diffusion method, and the inhibition zones were evaluated. It was observed that the antibacterial activity of flower honeys varied with strain and concentration, with a significant decrease at low concentrations. In contrast, chestnut honey exhibited a stronger, more consistent antibacterial effect against all tested bacterial strains, particularly at 100% concentration. The conclusion drawn from this research is that the floral origin of honey and applied concentration are decisive factors in antibacterial activity, and that chestnut honey has significant potential as a natural antibacterial agent.

Keywords: Antibacterial activity, Chestnut honey, Flower honey, Microorganisms

INTRODUCTION

Honey is a natural product produced by bees (*Apis mellifera*) from flower nectar, plant secretions, or excretions of plant-sucking insects, which are enzymatically transformed and matured within honeycombs. The chemical composition of honey varies significantly depending on the plant sources from which bees feed, as well as the geographical region and environmental conditions (Eteraf–Oskouei and Najafi 2013, Schneider et al. 2013). Honey is composed of approximately 80% carbohydrates, mainly glucose and fructose, along with smaller amounts of sucrose, maltose, isomaltose, trehalose, and melezitose, while the remaining 20% consists of water. In addition to these sugars, honey contains a variety of biologically active components, including vitamins, amino acids, enzymes, minerals, flavonoids, and phenolic acids. A substantial body of research has demonstrated the anti-inflammatory, wound-healing, antioxidant, immunomodulatory, and antineoplastic properties of honey (Bergman et al. 1983, Jaganathan et al. 2010, Swellam et al. 2003, Tonks et al. 2003). The focus of scientific interest has recently shifted to the antimicrobial properties of honey, particularly in light of increasing antibiotic resistance. A substantial body of research has documented the inhibitory effect of honey on various biological entities, including Gram-positive and Gram-negative bacteria, as well as certain types of yeast and fungi (Israili 2014, Vică et al. 2024). Previous studies have reported that the phenolic content of honey may vary depending on botanical origin, and polyfloral honeys may exhibit higher total phenolic compound levels than monofloral honeys (Kamel et al. 2023). However, it has been demonstrated that honey possesses substantial antimicrobial properties, effective against both Gram-positive and Gram-negative bacteria, as well as specific types of yeast and fungi. It has been established that the degree of antimicrobial efficacy exhibited by honey is contingent upon three factors: the floral provenance of the honey, its chemical composition, and the concentration of the substance employed. It is noteworthy that certain monofloral honeys, including Manuka honey, are distinguished by their broad-spectrum antibacterial properties (Albaridi 2019, Kačániová et al. 2022, Ogwu and Izah 2025).

The antimicrobial mechanisms of honey include low water activity due to high osmolarity, an acidic pH value, hydrogen peroxide (H_2O_2) production and phenolic compounds (Molan and Rhodes 2015). In diluted honey, hydrogen peroxide, produced by the glucose oxidase enzyme, has been reported to play an important role in antibacterial effects (Hossain et al. 2022). However, it has also been demonstrated that in some types of honey, the antibacterial activity originates from compounds independent of hydrogen peroxide (such as phenolic acids, flavonoids, proteins, peptides, and methylglyoxal). This phenomenon is particularly evident in Manuka and certain chestnut honeys, where it has been documented that the antibacterial activity persists even in the presence of catalase (Carter et al. 2016, Oryan et al. 2019). Another significant component of honey's antimicrobial effect is oligosaccharides. It has been reported that these compounds exhibit prebiotic properties, thereby supporting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and indirectly contributing to the suppression of pathogenic microorganisms (Burlando and Cornara 2013, Cornara et al. 2017). The extant literature also contains findings that honey is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin–resistant enterococci, and various Gram-negative pathogens. (Carter et al. 2016).

Chestnut honey is a monofloral honey characterised by its elevated phenolic compound content and distinctive biological activity. A number of studies have reported that chestnut honey exhibits an inhibitory effect on a range of microorganisms of clinical and environmental importance, including *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* (Almasaudi 2021, Fratianni et al. 2023, Grabek–Lejko and Worek 2024, Wadi 2022). This phenomenon is hypothesised to be attributable to the low pH value of the honey, its high viscosity, and the damage inflicted on cell membranes by its phenolic compounds.

The escalating interest in natural antimicrobial agents, driven by the mounting challenges posed by antibiotic resistance, underscores the imperative to evaluate honey as a prospective alternative or complementary antimicrobial agent. In this context, a comparative analysis of the antibacterial potential of honeys from diverse floral origins is imperative. The objective of this study is to compare the in vitro antibacterial activities of chestnut and flower honeys from diverse floral sources, with a focus on their efficacy against a range of significant pathogenic bacterial strains.

MATERIALS AND METHODS

Honey Samples

A total of 40 honey samples obtained from diverse floral sources in the Aegean and Marmara regions of Türkiye were evaluated in this study. The samples were collected from multiple provinces within these regions and were harvested in 2025. Information on the floral origin of the samples was based on producer/seller declarations; no melissopalynological (pollen) analysis was performed. The harvest year and regional origin of the samples were recorded and are reported herein. The samples comprised 20 of each type of honey: flower and chestnut. All honey samples were stored at room temperature (approximately 20–25°C) in a dark environment in airtight containers prior to analysis and were homogenised before the experiments. The analyses were performed within a short storage period (approximately 1–2 weeks to 1 month) to preserve their original physicochemical and antimicrobial properties.

Sterility Control

Before the antimicrobial analysis, the honey samples were subjected to microbiological sterility testing. One milliliter of each honey sample was aseptically transferred to Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) media using a sterile technique. MHA plates were subjected to an incubation period at 37°C for a duration of 24–48 hours, while SDA plates were incubated at 25°C for a period of 5 days. Samples exhibiting no visible microbial growth after incubation were utilised in the antibacterial tests.

Preparation of Honey Concentrations

The antibacterial activity of the honey samples was evaluated at 100% (undiluted) and 25% (v/v). The 25% solutions were prepared aseptically using sterile distilled water.

Bacterial Strains and Culture Conditions

The antibacterial activity of the honey samples was evaluated against the following standard reference strains:

Escherichia coli (*E. coli*) ATCC 43894

MRSA ATCC 43300

Staphylococcus aureus (*S. aureus*) ATCC 25923

Salmonella enterica serovar *Enteritidis (S. Enteritidis)* ATCC 13076

10 microlitres of standard strains were inoculated into Mueller-Hinton Broth (MHB) and incubated at 37°C for 24–48 hours. After incubation, the bacterial suspensions were adjusted to a 0.5 McFarland standard.

Agar Well Method

The antibacterial activity of the samples was determined by the agar well diffusion method. A volume of 1 mL of a bacterial suspension, having been adjusted to a standard of 0.5 McFarland, was applied in a uniform manner to the surface of Mueller-Hinton Agar (MHA). Wells with a diameter of 5 mm were created on the agar surface under sterile conditions. Subsequently, 100 µL of each honey sample was introduced into the wells. The plates (90 mm.) were then incubated at 37 °C for 24 hours. The diameters of the inhibition zones formed at the end of the incubation period were measured in millimetres (mm). Samples exhibiting zone diameters below 12 mm were considered to show no significant antibacterial activity. This threshold was not based on clinical antibiotic susceptibility breakpoints (e.g., CLSI/EUCAST) but was defined as an operational criterion that accounted for the inherent limitations of the agar well-diffusion method. In studies evaluating natural products such as honey, no universally accepted cutoff value exists for interpreting inhibition zones, as results are influenced by viscosity, diffusion capacity, and assay conditions. It has been reported that agar diffusion methods may show limited reliability for small inhibition zones due to diffusion constraints, and such zones are often interpreted as weak or negligible activity. Therefore, a 12 mm threshold was adopted to exclude minimal or ambiguous inhibition effects and to ensure a more robust interpretation of the results (Balouiri et al., 2016; Hossain et al., 2022).

Positive and Negative Controls

Negative Control: Sterile distilled water was applied to the wells at 100 µL, and its inhibitory effect was confirmed to be absent.

Positive control: The Kirby-Bauer disk diffusion method was used to determine bacterial susceptibility. Antibiotic disks appropriate for the identification of the microorganism in question were placed on Mueller-Hinton Agar, and the plates were then incubated at 37°C for a period of 24 hours. The resulting zone diameters were interpreted in accordance with standard evaluation criteria. Erythromycin (15 µg) discs were utilised as positive controls for Gram-positive strains, and trimethoprim-sulfamethoxazole (1.25/23.75 µg) discs were employed for Gram-negative strains. The selection of antibiotics was based on two key factors: first, the clinical significance of the microorganisms tested; and second, compliance with CLSI disk diffusion standards.

Antibiotic Susceptibility Test

The antibiotic susceptibilities of the tested bacterial strains were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar. Antibacterial activity was evaluated based on inhibition zone diameters. Zone diameters of ≤12 mm were considered indicative of no significant antibacterial activity. All agar well diffusion assays were performed in triplicate for each honey sample and bacterial strain combination, and inhibition zone diameters were measured independently for each replicate, as described in previous studies (Alshaybawee et al. 2025, Kumera et al. 2025, Petrović et al. 2024).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software. The antibacterial efficacy of the honey samples was evaluated by measuring the inhibition zone, which was categorised as "activity present" or "no activity". The chi-squared (χ^2) test was utilised to compare the antibacterial efficacy of flower and chestnut honeys at varying concentrations (100% and 25%). The statistical significance level was set at $p < 0.05$ for all analyses.

RESULTS

The present study investigates the *in vitro* antibacterial effects of chestnut and flower honeys from various provinces in Türkiye. The honeys were tested at 100% and 25% concentrations. The standard bacterial strains used were *S. aureus* ATCC 25923, *E. coli ATCC* 43894, MRSA ATCC 43300, and *S. Enteritidis* ATCC 13076.

An investigation was conducted into the antibacterial activity of various samples of flower honey. It was found that inhibition zones ranging from 20 to 35 mm were observed in nine out of twenty honey samples at a 100% concentration against the *S. aureus* (ATCC 25923) strain. However, no inhibition was observed in eleven samples. At a 25% concentration for the same strain, no inhibition zone was observed in 13 honey samples, while zones with a diameter of 20–35 mm were detected in 7 samples. For the *E. coli* (ATCC 43894) strain at 100% concentration, inhibition zones of 28–35 mm were observed in five honey samples. However, no antibacterial effect was observed in 15 samples. At a 25% concentration, zones of 19–25 mm were observed in only 5 samples, whereas 15 samples were negative (Figure 1).

In the case of the MRSA (ATCC 43300) strain, inhibition zones ranging from 15–30 mm were observed in 17 of 20 flower honey samples at a 100% concentration, while no inhibition was detected in 3 samples. At a 25% concentration, no inhibition zone was observed in any of the samples. In the case of *S. Enteritidis* (ATCC 13076), 21–32 mm zones were observed in 4 of the 20 honey samples examined at 100% concentration, while 16 samples were negative.

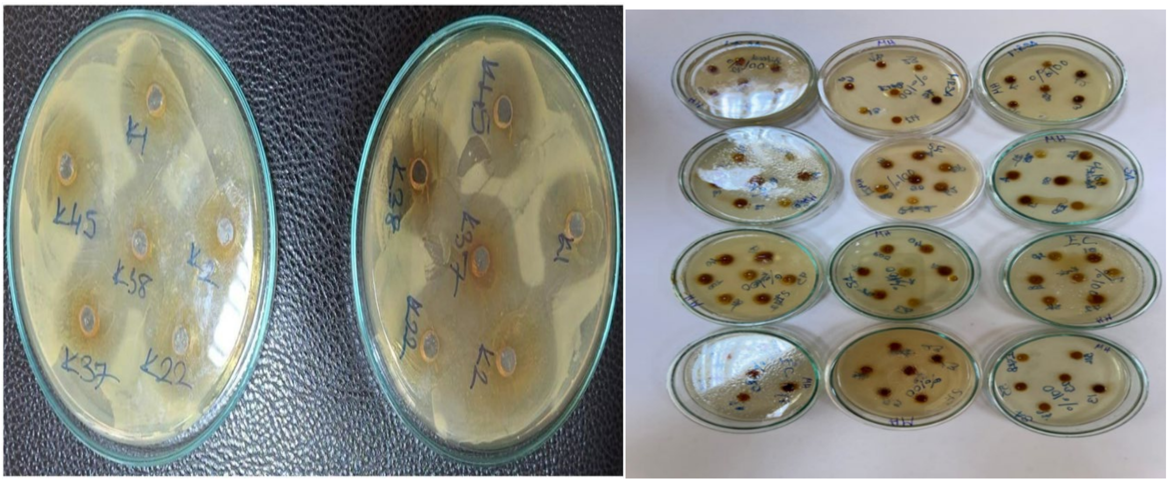

Figure 1. Comparative antibacterial activity of flower and chestnut honey samples against the tested bacterial strains at different concentrations, expressed as inhibition zone diameters (mm).

Table 1. Distribution of inhibition zone diameters (mm) produced by flower honey samples against tested bacterial strains at 100% and 25% concentrations.

Bacterial Strain (ATCC)	Concentration	Number of samples showing inhibition (n)	Diameter of inhibition zone (mm)
S. aureus 25923	100%	9	20–35
	25%	7	20–35
E. coli 43894	100%	5	28–35
	25%	5	19–25
MRSA 43300	100%	17	15–30
	25%	–	–
S. Enteritidis 13076	100%	4	21–32
	25%	4	20–25

In the course of the evaluation of the antibacterial efficacy of chestnut honey, inhibition zones ranging from 18–38 mm were formed in all 20 samples tested against *S. aureus* (ATCC 25923) at 100% concentration. At a concentration of 25%, a 22 mm zone was observed in a single sample, while no inhibition was detected in 19 samples. In the case of MRSA (ATCC 43300), 16–36 mm zones were formed in 18 samples at 100% concentration, while 2 samples were negative. In the samples examined, no inhibition was observed at 25% concentration. In the *S. Enteritidis* (ATCC 13076) strain, inhibition zones ranging from 18–32 mm were observed in all 20 samples tested at 100% chestnut honey, whereas no antibacterial effect was detected in any sample at 25%. In 19 samples, a 21–32 mm zone of inhibition was observed against the *E. coli* (ATCC 43894) strain at 100% concentration, while one sample exhibited no inhibition. In the samples at 25% concentration, no inhibition zone was observed (Table 2).

The antibacterial activity of both flower and chestnut honeys was concentration-dependent, with the highest inhibition observed at 100% concentration. The present study examined and compared the antibacterial efficacy of chestnut honeys with that of flower honeys. The results demonstrated that chestnut honeys exhibited a more pronounced and consistent antibacterial effect, particularly against strains of *S. aureus*, MRSA, *E. coli*, and *S. Enteritidis*. At a concentration of 25%, a marked and complete diminution in antibacterial activity was observed. Indeed, the activity was completely absent against certain strains.

Table 2. Distribution of inhibition zone diameters (mm) produced by chestnut honey samples against tested bacterial strains at 100% and 25% concentrations.

Bacterial strain (ATCC)	Concentration	Number of samples showing inhibition (n)	Diameter of inhibition zone (mm)
S. aureus 25923	100%	20	18–38
	25%	1	22
E. coli 43894	100%	18	16–36
	25%	–	–
MRSA 43300	100%	20	18–32
	25%	–	–
S. Enteritidis 13076	100%	19	21–32
	25%	–	–

A Statistical Analysis of the Antibacterial Efficacy of Flower and Chestnut Honeys

In the present study, the antibacterial efficacy of floral and chestnut honeys was compared statistically. The results demonstrated that chestnut honey exhibited significantly higher inhibition activity than floral honey at 100% concentration ($\chi^2 = 32.87$; $p < 0.001$). In chestnut honey samples, inhibition zones were observed against nearly all tested bacterial strains, whereas the activity of floral honey was more limited and heterogeneous. At 25% concentration, a general reduction in antibacterial activity was observed for both honey types; however, chestnut honey still showed significantly greater antibacterial effects compared to floral honey ($\chi^2 = 9.64$; $p = 0.002$). Overall, the findings indicate that antibacterial activity is influenced not only by concentration but also by floral origin, with chestnut honey consistently demonstrating higher activity than floral honey. SPSS–based chi-square analyses at the bacterial strain level further confirmed that chestnut honey exhibited significantly stronger antibacterial activity than floral honey at 100% concentration across all tested pathogens ($p < 0.05$), while at 25% concentration the activity varied depending on both bacterial strain and honey type.

DISCUSSION

In this study, the antibacterial effects of 100% and 25% concentrations of flower and chestnut honeys obtained from different floral sources on *E. coli* (ATCC 43894), MRSA (ATCC 43300), *S. aureus* (ATCC 25923) and *S. Enteritidis* (ATCC 13076) were evaluated using the agar diffusion method. The findings indicated that antibacterial activity was significantly concentration-dependent in both honey groups, with the 100% concentration producing larger inhibition zones compared to the 25% concentration. This is consistent with the literature, which suggests that honey's antimicrobial activity decreases with dilution.

The concentration-dependent antibacterial effect of honey has been widely reported in the literature. Almasaudi et al. (2017) reported that Manuka and other monofloral honeys exhibited a significant inhibitory effect against both methicillin-resistant and susceptible *S. aureus* strains at concentrations of 20% and 10% (w/w). Similarly, Tan et al. (2009) demonstrated that Manuka honey exhibited high antibacterial activity against *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*.

In studies on MRSA, 100% undiluted honey was reported to produce inhibition zones ranging from 11 to 18 mm (Ogbaje *et al*. 2006). Furthermore, it has been reported that various diluted concentrations (20–40% v/v) also inhibit MRSA growth (Ani et al. 2020). These findings are consistent with the strong antibacterial activity observed in our study, particularly at 100% concentration.

Honey exhibits antibacterial activity through multiple synergistic mechanisms, including high osmotic pressure caused by its sugar content, low pH, hydrogen peroxide production, and the presence of phenolic compounds (Hossain et al. 2022, Sagdıc et al. 2013). The high sugar concentration reduces water availability for microorganisms, while the honey's acidic nature inhibits the growth of many pathogenic bacteria. In addition, hydrogen peroxide generated by glucose oxidase activity contributes substantially to antimicrobial efficacy by inducing oxidative stress in bacterial cells. Previous studies have also reported that peroxide and non-peroxide components, including phenolic acids and flavonoids, collectively contribute to honey's antimicrobial properties (Hossain et al. 2022).

The antibacterial activity of honey may vary depending on its botanical origin and chemical composition. Floral source significantly influences the phenolic profile, antioxidant capacity, and biological activity of honey. Dark–colored monofloral honeys generally possess higher phenolic and flavonoid contents compared to many multifloral honeys, which may enhance their antimicrobial potential. Among monofloral honeys, chestnut honey is considered one of the varieties with strong antimicrobial activity due to its rich phenolic composition and high antioxidant capacity. These characteristics may explain the more pronounced antibacterial activity of chestnut honey observed in the present study against both Gram-positive and Gram-negative bacteria (Al–Kafaween et al. 2023, Hossain et al. 2022).

When evaluated in terms of Gram-negative bacteria, it is known that honey's effects may vary across species. Wilkinson et al. (2005) reported that various honey samples exhibited concentration–dependent inhibition against *E. coli* and *P. aeruginosa*, and that efficacy was lost at a concentration of 1%. In this study, too, the significant reduction in zone diameters at a 25% concentration can be explained by honey's hyperosmotic nature, low pH, and the inability of its phenolic compounds to generate sufficient antimicrobial pressure below the threshold concentration.

Similar results have been obtained in studies on *Salmonella* species. Meuller et al. (2014) reported that honey concentrations in the 2–10% range exhibited an inhibitory effect on *S. Enteritidis*. Our findings support the notion that applying honey, particularly at high concentrations, significantly inhibits Salmonella.

The present findings showed that both raw honey and honey-herbal mixtures exhibited significant antibacterial activity against *Staphylococcus aureus* and MRSA. Undiluted samples produced greater inhibition zones than diluted samples, indicating concentration-dependent antibacterial effects. However, lower activity was observed against *Pseudomonas aeruginosa*. These results suggest that honey, alone or combined with herbal additives, can serve as a promising natural antibacterial agent, particularly against Gram-positive bacteria (Elk-herbawy et al. 2024)

Chestnut honey is among the monofloral honeys with strong antimicrobial properties, owing to its high phenolic content and antioxidant capacity. A recent study conducted in Bosnia and Herzegovina demonstrated that chestnut honey produces significant inhibition zones against both Gram-positive and Gram-negative bacteria (Čengić et al. 2025). Acacia honey, acacia comb honey, floral and manuka honey expressed higher antibacterial activity against Gram-positive bacteria. Chestnut honey showed higher antibacterial activity against Gram-negative bacteria, particularly against *S. Enteritidis* and *S. Typhimurium*. All tested honeys showed the strongest antibacterial effect on S. group D. Acacia honey and acacia honey comb did not show antibacterial activity against the clinical isolate of *S. aureus*. The best antimicrobial effect was observed with manuka and flower honey, which showed the strongest bacteriostatic effect, while chestnut honey showed the strongest bactericidal effect (Kalaba et al. 2020). Similarly, Güneş (2021) reported that 100% chestnut honey exhibited a pronounced antibacterial effect against *S. Typhimurium* and *E. coli* O157:H7, with efficacy decreasing at a 25% concentration (Güneş 2021). Another study conducted in the Bursa region also reported that 100% chestnut honey exhibited high activity against *S. aureus* and *E. faecalis*, with zone diameters decreasing as the concentration decreased (Borum 2016).

These findings are consistent with our study, which showed that chestnut honey exhibits more pronounced antibacterial activity, particularly at 100% concentration.

In our study, the antibacterial efficacy of chestnut honey was compared with that of various flower honeys, and chestnut honey showed higher inhibitory activity against all pathogens examined than the flower honeys. Whilst this finding is partly consistent with the general trends reported in the literature, it presents unique results that highlight the influence of honey’s botanical source.

In both types of honey, antibacterial activity increased in direct proportion to concentration. Chestnut honey exhibited a distinct inhibitory effect even at low concentrations; in floral honeys, however, activity remained limited at low concentrations. This indicates that the bioactive components of honey are concentration-dependent and exhibit specificity for the type of microorganism.

Conclusion

In conclusion, the present study demonstrated that chestnut honey exhibited stronger antibacterial activity than flower honey against the tested bacterial strains, including *Staphylococcus aureus*, MRSA, *E. coli*, and *S. Enteritidis*. This higher antibacterial activity may be associated with the rich phenolic and flavonoid content of chestnut honey, together with other biologically active compounds.

Furthermore, antibacterial activity in both honey types was concentration-dependent, with significantly greater inhibition at 100% concentration. These findings indicate that the botanical origin of honey plays an important role in determining its antibacterial potential. Overall, chestnut honey may be considered a promising natural antibacterial agent and a potential complementary approach for controlling certain pathogenic microorganisms.

Artificial Intelligence (AI) Use Declaration: No AI tools were used in writing, editing, analyzing, or generating any part of the manuscript text. The study and manuscript were prepared without the use of artificial intelligence tools.

Author Contributions: AEB contributed to the study conception and design, project planning, sample collection, laboratory analyses, interpretation of the results, and manuscript writing. MK contributed to the laboratory analyses. AG, FFA, and MV were involved in sample collection. The manuscript was written by AEB. All authors read and approved the final manuscript.

Conflict of Interest: I declare no conflict of interest.

Data Availability Statement: The data supporting the findings of this study are available from me upon reasonable request.

Ethical Approval: Ethical approval for this study was obtained from the Ethics Committee of Balıkesir University (Approval Date: May 22, 2025; Decision No: 2025/5-2)

Funding Source: This work was supported by the TÜBİTAK 2209/B Project

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