



## INVESTIGATION OF THE ANTIMICROBIAL EFFECTS OF TRADITIONALLY PREPARED VINEGARS FROM *Plantago Major L.* AND *Hypericum Perforatum L.* COLLECTED IN ORDU PROVINCE ON SELECTED PATHOGENIC MICROORGANISMS

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**Abstract:** In this study, the antimicrobial effects of traditionally fermented vinegars produced from *Plantago major* L. (common plantain) and *Hypericum perforatum* L. (St. John's Wort), collected from the Ünye district of Ordu Province, Türkiye, were evaluated. The vinegar samples were tested against 11 pathogenic microorganisms, including Gram-positive and Gram-negative bacteria as well as *Candida* species, using the agar disk diffusion method. The results showed that both types of vinegar exhibited significant antimicrobial activity, particularly at undiluted (1:1) and 1/2 dilutions. The highest antibacterial effect for *P. major* vinegar was observed against *Proteus vulgaris* (15.33±0.33 mm). Antifungal activity, however, was generally lower compared to bacterial inhibition. The pH values of *P. major* and *H. perforatum* vinegars were measured as 2.62 and 2.72, respectively, indicating strong acidity, which likely contributes to their antimicrobial properties. These findings suggest that vinegars produced from medicinal plants through traditional fermentation methods have the potential to serve as natural antimicrobial agents. Further biochemical studies are recommended to explore their potential applications in pharmaceutical and food safety fields.

**Keywords:** *Plantago major* L., *Hypericum perforatum* L., Antimicrobial activity, Disk diffusion

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Received: July 23, 2025

Accepted: October 24, 2025

Published: November 15, 2025

**Cite as:** Veyisoglu A, Tatar D, Tepekoz I. 2025. Investigation of the antimicrobial effects of traditionally prepared vinegars from *Plantago major* L. and *Hypericum perforatum* L. collected in Ordu Province on selected pathogenic microorganisms. BSJ Eng Sci, 8(6): 1936-1942.

### 1. Introduction

In recent years, interest in natural-origin products has significantly increased, particularly due to the adverse effects associated with long-term use of synthetic drugs and the growing problem of antibiotic resistance. In this context, functional foods and plant-based products with antimicrobial potential have emerged as promising alternatives for treatment and preventive health measures. Vinegar is a natural product widely consumed in both traditional and modern medicine due to its various pharmacological effects, drawing attention for its antimicrobial, antioxidant, and metabolic regulatory properties (Johnston and Gaas, 2006; Nassiri-Asl and Hosseinzadeh, 2016; Ashchyan et al., 2018).

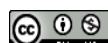
The antimicrobial activity of vinegar is primarily attributed to its acetic acid content and other organic acids. Acetic acid creates a low pH environment and disrupts microbial cell membranes, thereby inhibiting the proliferation of pathogenic microorganisms (Zhang et al., 2020). Moreover, the development of non-antibiotic antimicrobial agents holds potential to reduce antibiotic usage in infectious disease treatment and to limit the spread of antibiotic resistance (Carson et al., 2017;

Antoniewicz et al., 2021).

*Plantago major* L. (common plantain) is often regarded as a wild weed; however, it has been widely used in traditional medicine due to its potent antioxidant and immunomodulatory properties (Liu et al., 2002). Literature reports indicate its application in supportive treatment of various health conditions, including skin diseases, gastrointestinal disorders, respiratory infections, urinary tract infections, hemorrhoids, pain, and fever (Samuelson, 2000; Velasco-Lezama et al., 2006). Additionally, transdermal patches containing *P. major* extract have been reported to reduce nicotine cravings (Cody, 1998; Stanisavljević et al., 2008).

*Hypericum perforatum* L. (St. John's Wort), belonging to the *Hypericaceae* family, is a perennial plant of significant medicinal value. It is traditionally used primarily for neuropsychiatric disorders such as depression, and it also exhibits notable anti-inflammatory, antiviral, and antimicrobial activities. Recent studies have demonstrated its inhibitory effects against various bacterial and fungal pathogens (Saddique et al., 2010).

Vinegar production involves the fermentation of fermentable sugars first into ethanol by yeasts, followed by oxidation of ethanol to acetic acid by acetic acid



bacteria (AAB). The quality of vinegar is influenced by the raw materials used, the microbial flora involved, and the production method. In addition to these factors, naturally fermented vinegars contain not only acetic acid but also bioactive compounds such as vitamin B1, nicotinic acid, and other metabolites, which enhance their biofunctional properties (Elhan, 2014).

This study aims to evaluate the antimicrobial activities of vinegars produced by traditional fermentation methods from *Plantago major* L. and *Hypericum perforatum* L. plants collected from Ordu province against eleven different pathogenic microorganisms. Although antimicrobial activities of the essential oils and extracts of these plants have been previously investigated, there is insufficient evidence on the antimicrobial potential of vinegars produced via traditional methods. This study seeks to contribute to the development of novel natural antimicrobial agents by integrating ethnobotanical knowledge with contemporary biochemical analyses.

## 2. Materials and Methods

*P. major* L. (common plantain) and *H. perforatum* L. (St. John's Wort) were collected in June 2025 from natural habitats in the Ünye district of Ordu Province, Türkiye. The fresh plant materials were thoroughly washed with distilled water to remove surface contaminants. No drying process was applied, and the plants were used fresh for vinegar production. A total of eleven pathogenic microorganisms were used to evaluate the antimicrobial activity of the vinegar samples. The Gram-positive bacterial strains included *Bacillus subtilis* ATCC 6633, *Brevibacillus brevis* ATCC 35690, *Listeria monocytogenes* NCTC 5348, and *Staphylococcus aureus* ATCC 25923. The Gram-negative bacterial strains were *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* CCU 2531, *Proteus vulgaris* FMC 1, and *Salmonella typhimurium* NRRLE 4413. The fungal strains tested included *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 15126, and *Candida tropicalis* ATCC 13803. All strains were obtained from standard microbial culture collections and prepared under appropriate laboratory conditions for antimicrobial testing.

Vinegar production was carried out using traditional fermentation methods. Washed, fresh plant materials were combined with potable water, and 5% (v/v) natural honey was added as a carbon source to promote fermentation. The mixture was placed in glass containers, loosely covered to allow airflow, and left to ferment spontaneously at room temperature (~25 °C) for approximately 30 days. Upon completion of fermentation, the resulting vinegars were filtered, bottled under aseptic conditions, and transported to the laboratory. All vinegar samples were stored at +4 °C until microbiological analyses were performed. The pH of the *P. major* and *H. perforatum* vinegars was measured using a calibrated digital pH meter and the measurements were performed in triplicate to ensure accuracy. The antimicrobial properties of traditionally fermented

vinegars produced from *Plantago major* L. and *Hypericum perforatum* L. were evaluated using the agar disk diffusion method, as modified from standard protocols described by Collins et al. (1989), Taner Saracoğlu (2024), and Veyisoğlu et al. (2024a; 2024b). Bacterial strains were cultivated on Mueller- Hinton Agar (MHA), and fungal strains were maintained on Sabouraud Dextrose Agar (SDA; Difco). All microbial suspensions were adjusted to the 0.5 McFarland turbidity standard to ensure consistency in inoculum density.

Each standardized suspension (100 µL) was uniformly spread across the surface of the corresponding agar plates using sterile cotton swabs. The plates were then allowed to dry for approximately 5 minutes under laminar airflow to ensure absorption and even distribution.

The vinegar samples, obtained through traditional fermentation of fresh, unprocessed *P. major* and *H. perforatum* plants, were tested at different concentrations to determine their dose-dependent antimicrobial effects. Initially, 2000 µL of undiluted vinegar was placed into the first sterile container. For subsequent dilutions, 1000 µL of sterile distilled water was added to each of the remaining containers, followed by serial dilution using a micropipette, resulting in four concentrations: undiluted (1:1), 1:2, 1:4, and 1:8 (v/v). From each dilution, 25 µL was aseptically pipetted onto sterile 6 mm blank paper discs. These discs were then gently placed on the inoculated agar surfaces. The plates were left to dry under sterile airflow for about 5 minutes prior to incubation.

Bacterial cultures were incubated at 37 °C for 24 hours, whereas fungal cultures were incubated at 28 °C for durations optimized according to the specific growth characteristics of each species. Sterile distilled water was employed as the negative control. Rifampicin and nalidixic acid served as positive controls for the bacterial strains, while nystatin and cycloheximide were used as positive controls for the fungal strains.

In bacterial tests, rifampicin (10 µg/mL) and nalidixic acid (10 µg/mL) were used as positive controls. Rifampicin inhibits RNA polymerase, exhibiting a potent and rapid effect on Gram-positive species, and is also effective against some Gram-negative bacteria. Nalidixic acid, on the other hand, targets DNA gyrase/topoisomerase, exhibiting activity specifically against Gram-negative enteric bacteria. Therefore, two antibiotics were selected as complementary standards representing different mechanisms of action. In fungal tests, nystatin (NS100) and cycloheximide (50 µg/mL) were used as positive controls. Nystatin binds to ergosterol in the cell membrane, disrupting permeability, while cycloheximide inhibits protein synthesis in eukaryotic ribosomes. Thus, reference agents that provide both membrane-targeting and ribosomal inhibition were used in the fungal tests. The selection of these four agents together allowed for reliable comparisons of the effects of the tested vinegar samples.

After incubation, antimicrobial activity was assessed by measuring the diameters of the inhibition zones (including disc diameter) in millimeters, using a digital caliper for accuracy. The tests were done in triplicate. Data storage analysis for our study was conducted using SPSS 20 (IBM). Group comparisons were made using one-way analysis of variance (ANOVA) and Tukey HSD test. Inhibitory zone diameters (mm) were measured for each amount, and the values were expressed as arithmetic mean  $\pm$  standard error (SE) (Genç and Soysal, 2018).

### 3. Results

The inhibition zone diameters of the vinegar samples against the tested microorganisms are summarized in Table 2. The pH values of the vinegar samples obtained from *P. major* and *H. perforatum* were measured as 2.62 and 2.72, respectively. These results demonstrate that both vinegars exhibit a strongly acidic nature, which is consistent with previously reported values for traditionally fermented herbal vinegars. The low pH values are primarily attributed to the presence of organic acids, particularly acetic acid, formed during the fermentation process.

**Table 1.** pH results of the vinegar samples

	pH
<i>P. major</i> vinegar	2.62
<i>H. perforatum</i> vinegar	2.72

As shown in Table 2, the antimicrobial activities of *P. major* and *H. perforatum* vinegars against bacterial and fungal microorganisms were evaluated at various concentrations. In general, it was observed that both vinegar types, especially at high concentrations (undiluted), formed significant inhibition zones against Gram-positive and Gram-negative bacteria.

The obtained data were analyzed using one-way analysis of variance (One-Way ANOVA). When a significant difference was found at  $P < 0.05$  in the ANOVA, the Tukey HSD (honestly significant difference) multiple comparison test was applied to determine the concentrations that accounted for the difference.

The results indicate that there is no statistical difference between concentrations sharing the same letter in the same row; different letters (a, b, c, etc.) indicate a significant difference ( $P < 0.05$ ).

*P. major* vinegar exhibited inhibition zones of up to  $12.33 \pm 0.33^a$  mm against Gram-positive bacteria such as *B. subtilis* (BS), *B. brevis* (BB), *S. aureus* (SA), and *L. monocytogenes* (LM). Among Gram-negative bacteria, *P. major* vinegar exhibited the highest activity at  $15.33 \pm 0.33^a$  mm against *P. vulgaris* (PV) (Table 2, 3).

*H. perforatum* vinegar exhibited similar inhibition values  $10.33 \pm 0.33^a$  mm, but showed slightly lower activity against some bacteria compared to *P. major* vinegar (Table 2, 3; Figure 1).

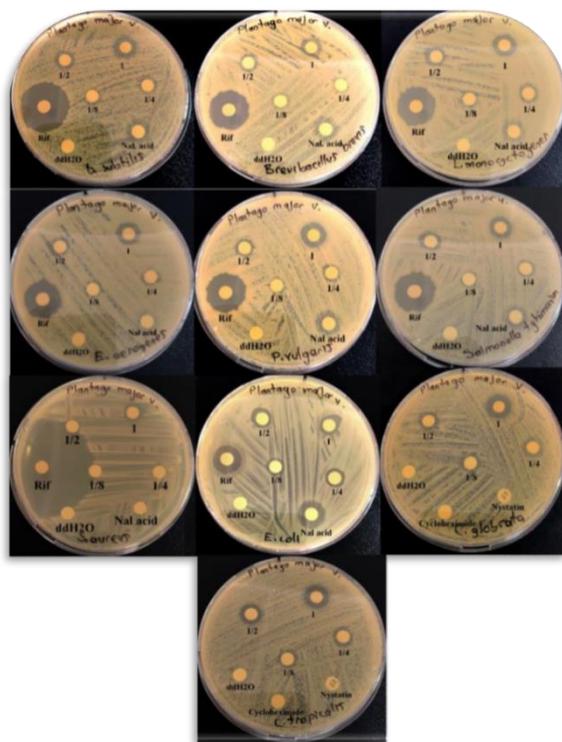
Both vinegars showed a significant decrease in

antimicrobial activity as their concentrations decreased (1/2, 1/4, and 1/8 dilutions). It is particularly noteworthy that the zones of inhibition generally became smaller or disappeared completely at the 1/8 dilution level. This demonstrates that antimicrobial activity is dose-dependent (Table 2, 3; Figure 2).

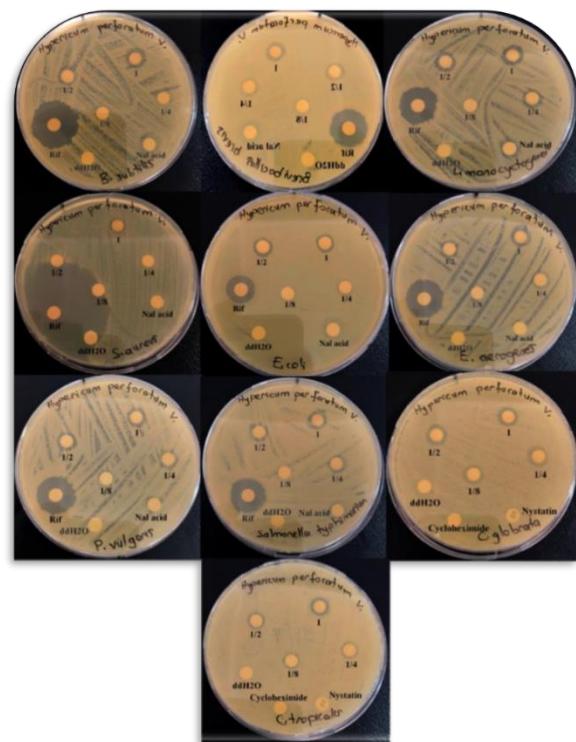
When evaluated against fungal strains, both vinegars demonstrated some activity against *C. albicans* (CA), *C. glabrata* (CG), and *C. tropicalis* (CT), particularly at undiluted and 1/2 concentrations, but overall exhibited lower activity compared to standard positive controls such as rifampicin, nalidixic acid, nystatin, and cycloheximide. No inhibition was observed with ddH<sub>2</sub>O, used as a negative control (Table 2, 3; Figure 2).

When comparing positive controls, rifampicin and nalidixic acid demonstrated the expected high antimicrobial activity against bacteria, while nystatin and cycloheximide demonstrated specific effects against fungal strains. This supports the validity of the experiment and the reliability of the methods used (Table 2, 3; Figure 2).

In a 2017 study, Bakir et al. (2017) examined the antimicrobial properties of 18 different vinegar types—including those made from apple, grape, pomegranate, balsamic, blueberry, rosehip, viburnum, lemon, blackberry, hawthorn, artichoke, mulberry, Arabian date, apricot, and rice—against *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *Salmonella typhimurium* ATCC 14028 using the disc diffusion technique. Among the tested apple vinegar samples, one with a 2.12% acidity level produced inhibition zones of  $11 \pm 1$  mm for both *S. aureus* and *E. coli*, while another with a higher acidity of 5.02% yielded slightly smaller zones  $9 \pm 1$  mm for *S. aureus* and  $10 \pm 2$  mm for *E. coli*. The vinegars were grouped based on their acidity into five ranges: 0.5–1%, 1–2%, 2–3%, 3–5%, and 5–7%. The inhibition zones recorded for *S. aureus* within these groups varied between 8.75 mm and 13 mm. However, an increase in acidity did not correspond to a linear increase in antimicrobial activity. This inconsistency suggests that other variables—such as the source ingredients of the vinegar or differences in pH—might also significantly contribute to their antibacterial effectiveness.



**Figure 1.** Inhibition zones (mm) formed by different concentrations (undiluted, 1/2, 1/4, 1/8) of *P. major* vinegar against 10 pathogenic microorganisms.



**Figure 2.** Inhibition zones (mm) formed by different concentrations (undiluted, 1/2, 1/4, 1/8) of *H. perforatum* vinegar against 10 pathogenic microorganisms.

**Table 2.** Antimicrobial activity of *P. major* and *H. perforatum* vinegars

<i>P. major</i> vinegar	Gram-positive bacteria			
	BS	BB	SA	LM
1	11.67±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	7.33±0.33 <sup>a</sup>	12.33±0.33 <sup>a</sup>
1/2	10.33±0.33 <sup>a</sup>	8.33±0.33 <sup>b</sup>	-	10.33±0.33 <sup>b</sup>
1/4	7.33±0.33 <sup>b</sup>	-	-	9.67±0.33 <sup>b</sup>
1/8	-	-	-	-
Rifampicin (10 µg/ml)	25.33 ± 0.33 <sup>a</sup>	21.00 ± 0.58 <sup>a</sup>	50.33 ± 0.33 <sup>a</sup>	21.00 ± 0.58 <sup>a</sup>
Nalidixic acid (10µg/ml)	10.33 ± 0.33 <sup>b</sup>	10.33 ± 0.33 <sup>b</sup>	-	12.33 ± 0.33 <sup>b</sup>
Nystatin (NS100)	ND	ND	ND	ND
Cycloheximide (50 µg/ml)	ND	ND	ND	ND
ddH <sub>2</sub> O	-	-	-	-
<i>H. perforatum</i> vinegar				
1	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>
1/2	9.00±0.58 <sup>a</sup>	9.00±0.58 <sup>a</sup>	-	10.33±0.33 <sup>a</sup>
1/4	-	9.00±0.58 <sup>a</sup>	-	9.00±0.58 <sup>a</sup>
1/8	-	-	-	-
Rifampicin (10 µg/ml)	25.33 ± 0.33 <sup>a</sup>	21.00 ± 0.58 <sup>a</sup>	50.33 ± 0.33 <sup>a</sup>	21.00 ± 0.58 <sup>a</sup>
Nalidixic acid (10 µg/ml)	10.33 ± 0.33 <sup>b</sup>	10.33 ± 0.33 <sup>b</sup>	-	12.33 ± 0.33 <sup>b</sup>
Nystatin (NS100)	ND	ND	ND	ND
Cycloheximide (50 µg/ml)	ND	ND	ND	ND
ddH <sub>2</sub> O	-	-	-	-

a, b, c= there is no statistical difference between concentrations sharing the same letter in the same row; different letters (a, b, c, etc.) indicate a significant difference ( $p < 0.05$ ). BS= *Bacillus subtilis* ATCC 6633, BB= *Brevibacillus brevis* ATCC 35690, SA= *Staphylococcus aureus* ATCC 25923, LM= *Listeria monocytogenes* NCTC 5348.

In the study conducted by Şengün and Kılıç (2018), the antimicrobial activity of homemade and commercial mulberry vinegars was investigated. The study tested the strains *Escherichia coli* O157:H7 ATCC 43895, *Listeria monocytogenes* Scott A, *Salmonella typhimurium* NRRLB 4420, *Staphylococcus aureus* 6538P, *Bacillus subtilis* ATCC 6037, *Escherichia coli* ATCC 1103, *Enterococcus faecalis* ATCC 29212, and *Pediococcus acidilactici* ATCC 8042 using the disk diffusion method. Homemade vinegar did

not produce inhibition zones against *S. aureus* and *E. coli* ATCC 1103, while commercial vinegar produced inhibition zones of 11.5 mm against *S. aureus* and 7.5 mm against *E. coli* ATCC 1103. It was concluded that the higher antibacterial effect of commercial mulberry vinegar was due to its higher acid content. Additionally, *E. coli* was identified as the most resistant microorganism against both types of vinegar.

**Table 3.** Antimicrobial activity of *P. major* and *H. perforatum* vinegars

<i>P. major</i> vinegar	Gram-negative bacteria				Fungus		
	EA	EC	PV	ST	CA	CG	CT
1	12.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	15.33±0.33 <sup>a</sup>	12.33±0.33 <sup>a</sup>	-	12.33±0.33 <sup>a</sup>	14.33±0.33 <sup>a</sup>
1/2	10.33±0.33 <sup>b</sup>	10.33±0.33 <sup>a</sup>	9.33±0.33 <sup>b</sup>	10.33±0.33 <sup>b</sup>	-	10.33±0.33 <sup>b</sup>	11.33±0.33 <sup>b</sup>
1/4	9.67±0.33 <sup>b</sup>	9.00±0.58 <sup>a</sup>	9.00±0.58 <sup>b</sup>	7.00±0.58 <sup>c</sup>	-	9.33±0.33 <sup>b</sup>	10.33±0.33 <sup>b</sup>
1/8	7.33±0.33 <sup>c</sup>	7.00±0.58 <sup>b</sup>	-	-	-	7.33±0.33 <sup>c</sup>	8.33±0.33 <sup>c</sup>
Rifampicin (10 µg/ml)	17.00±0.58 <sup>a</sup>	15.33 ±0.33 <sup>a</sup>	18.33±0.33 <sup>a</sup>	20.33 ±0.33 <sup>a</sup>	ND	ND	ND
Nalidixic acid (10 µg/ml)	-	13.33 ±0.33 <sup>b</sup>	10.33±0.33 <sup>b</sup>	12.33 ±0.33 <sup>b</sup>	ND	ND	ND
Nystatin (NS100)	ND	ND	ND	ND	30.33 ±0.33 <sup>a</sup>	-	-
Cycloheximide (50 µg/ml)	ND	ND	ND	ND	-	-	-
ddH <sub>2</sub> O	-	-	-	-	-	-	-
<i>H. perforatum</i> vinegar							
1	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	-	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>
1/2	9.00±0.58 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	10.33±0.33 <sup>a</sup>	-	10.33±0.33 <sup>a</sup>	8.33±0.33 <sup>b</sup>
1/4	9.00±0.58 <sup>a</sup>	-	10.33±0.33 <sup>a</sup>	8.33±0.33 <sup>b</sup>	-	10.33±0.33 <sup>a</sup>	8.33±0.33 <sup>b</sup>
1/8	-	-	-	-	-	8.33±0.33 <sup>b</sup>	8.33±0.33 <sup>b</sup>
Rifampicin (10 µg/ml)	17.00±0.58 <sup>a</sup>	15.33 ±0.33 <sup>a</sup>	18.33±0.33 <sup>a</sup>	20.33 ±0.33 <sup>a</sup>	ND	ND	ND
Nalidixic acid (10 µg/ml)	-	13.33 ±0.33 <sup>b</sup>	10.33±0.33 <sup>b</sup>	-	ND	ND	ND
Nystatin (NS100)	ND	ND	ND	ND	30.33 ± 0.33 <sup>a</sup>	-	-
Cycloheximide (50 µg/ml)	ND	ND	ND	ND	-	-	-
ddH <sub>2</sub> O	-	-	-	-	-	-	-

a, b, c= there is no statistical difference between concentrations sharing the same letter in the same row; different letters (a, b, c, etc.) indicate a significant difference ( $p < 0.05$ ). EA= *Enterobacter aerogenes* CCU 2531, EC= *Escherichia coli* ATCC 25922, PV= *Proteus vulgaris* FMC 1, ST= *Salmonella typhimurium* NRRLE 4413; CA= *Candida albicans* ATCC 10231, CG= *Candida glabrata* ATCC 15126, CT= *Candida tropicalis* ATCC 13803. ND: not determined, (-)= no inhibition zone was shown.

Similarly, in the study by Kara et al. (2021), the antimicrobial effects of various vinegar samples prepared using different methods and from different apple varieties were evaluated against five bacterial strains isolated from clinical samples (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* (ATB:57), *Escherichia coli* (ATB:97), and *Pseudomonas aeruginosa*) (114). The different vinegars produced inhibition zones ranging from 10.70 to 20.70 mm against *S. aureus* and from 9 to 20.70 mm against *E. coli*. Avcu (2023) investigated the antimicrobial activity of vinegar, povidone iodine and boric acid against *S. aureus* and *E. coli* strains isolated from the wound site. As a result of the study, it was determined that povidone iodine showed the highest antimicrobial effect against the

strains isolated from the wound site and boric acid showed the least antimicrobial effect. It was stated that vinegar can be an alternative to povidone iodine and boric acid as antibacterial.

In a comparable study, Hindi (2013) investigated the antimicrobial activity of apple cider vinegar alone and in combination with garlic against bacterial strains isolated from clinical samples, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Proteus vulgaris* and *Acinetobacter* species. The study demonstrated that the apple cider vinegar-garlic mixture

exhibited significantly stronger antimicrobial effects across all tested microorganisms compared to apple cider vinegar alone. The inhibition zones produced by the mixture ranged from 25 to 50 mm, whereas apple cider vinegar alone showed inhibition zones between 6 and 15 mm. Among Gram- positive bacteria, apple cider vinegar showed the largest inhibition zone against *S. aureus* (15 mm). For *E. coli*, the inhibition zone was measured at 9 mm.

When the effects of *P. major* vinegar on bacteria were examined, significant concentration-dependent differences were observed among all microorganisms ( $P<0.05$ ). According to the Tukey HSD test, concentrations of 1 and 1/2 produced the highest inhibition, and the zone diameter statistically decreased as the dilution ratio increased. For example, the mean zone diameter for *Escherichia coli* was  $10.33 \pm 0.33^a$  mm, while it decreased to  $7.00 \pm 0.58^b$  mm at the 1/8 concentration. Similarly, for *Candida tropicalis* and *C. glabrata*, the letter sequence "a > b > c" indicates that the effect is dose-dependent. These results demonstrate that *P. major* vinegar has strong antimicrobial potential, especially at high concentrations. Similar trends were observed in *H. perforatum* vinegar. Zone diameters were higher at 1/2 and 1/2 concentrations, but significantly lower at 1/4 and 1/8 dilutions ( $P<0.05$ ). For example, the zone diameter for *Candida glabrata*, which was  $10.33 \pm 0.33^a$  mm, decreased to  $8.33 \pm 0.33^b$  mm at 1/8 concentration. This indicates that the antimicrobial activity of vinegar is sensitive to dilution.

The findings obtained in this study reveal that vinegar samples exhibit stronger antimicrobial activity, particularly against bacteria. One of the main reasons for this is the cell structural differences between bacteria and fungi. The thick peptidoglycan layer of Gram-positive bacteria and the outer membrane structure of Gram-negative bacteria show a certain sensitivity to low pH and organic acids (Carson et al., 2017; Antoniewicz et al., 2021). On the other hand, fungi have an ergosterol-containing plasma membrane and a cellulose/chitin-based cell wall. These structures provide higher tolerance to acidic conditions, and therefore, the effect of vinegar samples against fungal species is more limited compared to bacteria (Saddiqe et al., 2010). Similarly, the literature reports that organic acids disrupt membrane integrity in bacterial cells and affect proton balance, but this effect is weaker in fungi (Zhang et al., 2020). Therefore, the observed dose-dependent reduction was more pronounced in bacteria, while it was partially limited in fungi due to higher resistance.

#### **4. Conclusion**

In conclusion, *P. major* and *H. perforatum* vinegars exhibit significant antimicrobial activity against both Gram- positive and Gram-negative bacteria, especially at high concentrations. However, their activity against fungi is more limited. This may be explained by the synergistic effect of the organic acids and plant components

contained in the vinegar. Furthermore, the decrease in efficacy with decreasing doses highlights the importance of appropriate dosage in the therapeutic applications of these natural products. Both vinegar types exhibited significant antimicrobial activity against the tested microorganisms. ANOVA and Tukey HSD results revealed that the inhibition zones became significantly smaller as the vinegar concentration decreased. Therefore, both herbal vinegars exhibited dose-dependent antimicrobial activity; these findings scientifically support the microbial inhibition properties reported in their traditional use.

#### **Author Contributions**

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	A.V.	D.T.	I.T.
C	50	40	10
D	60	30	20
S	60	20	20
DCP	50	50	-
DAI	40	30	30
L	70	30	-
W	50	50	-
CR	70	30	10
SR	80	-	20
PM	20	10	80
FA	50	40	10

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

#### **Conflict of Interest**

The authors declared that there is no conflict of interest.

#### **Ethical Consideration**

Ethics committee approval was not required for this study because there was no study on animals or humans.

#### **Acknowledgements**

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under the 2209-A Research Project Program. The authors would like to thank TÜBİTAK for its support.

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