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ORIGINAL RESEARCH PAPER

ANTIMICROBIAL ACTIVITY OF DIFFERENT KINDS OF TRADITIONAL VINEGAR AND ITS RELATIONSHIP WITH ANTIOXIDANT PROPERTIES

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Abstract

Vinegar is obtained from fruit and various vegetable sources by ethanol and acetic acid fermentation. Different production methods are used in vinegar production. Traditional vinegar has higher quality than industrial vinegar because it contains high amounts of bioactive compounds. When the results obtained in this study are examined, it can be concluded that the antioxidant, phenolic and organic acid content is effective on the antimicrobial effect of vinegar. The vinegar with the highest antimicrobial activity was determined as pomegranate vinegar against the gram-positive microorganism group and apple vinegar against the gram-negative microorganism group in this research.

The purpose of this study was to determine the antimicrobial activity of different kinds of vinegar (apple vinegar, grape vinegar, hawthorn vinegar, sour cherry vinegar, pomegranate vinegar) produced with the traditional method on some important foodborne pathogens (*Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Campylobacter jejuni)* by using antimicrobial disc diffusion method. Besides, the examination of organic acid content, antioxidant capacity and phenolic compounds of the vinegar, also their relationship with the antimicrobial effect was examined.

Keywords: sour cherry vinegar, pomegranate vinegar, hawthorn vinegar, antimicrobial effect, disc diffusion assay, organic acids

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Introduction

Vinegar, a plant-based food, draws attention as an important natural antimicrobial substance thanks to its organic acid content, phenolic compounds and essential oils. It is obtained from fruit and various vegetable sources by ethanol and acetic acid fermentation (Budak and Güzel-Seydim, 2010). The antimicrobial properties attributed to vinegar are mainly due to the acetic acid content. Bacteria are more sensitive to acetic acid than mold and yeast. Bacteria, which can grow above pH 6.0, are more effectively inhibited by applying acetic acid. Acid passes through the cell membrane of living organisms, causing cell death (Budak *et al.*, 2014; Sengun and Kilic, 2018).

Today, there are two types of vinegar in the market, traditional and industrial. Traditional vinegar has higher quality than industrial vinegar because it contains high amounts of bioactive compounds (Özen *et al.*, 2020). The type of the bioactive compounds and their amounts in vinegar are important parameters due to their direct relationship with the antimicrobial activity of vinegar (Sengun and Kilic, 2018).

In previous studies, the antimicrobial properties of various vinegar were evaluated such as mulberry (Aydın, 2013; Sengun and Kilic, 2018), grape, apple and pomegranate (Duru, 2016; Kelebek *et al.*, 2017), blueberry and honey (Fonseca *et al.*, 2018), balsamic, rosehip, gilaburu, lemon, artichoke, apricot, hawthorn, persimmon, rice (Bakir *et al.*, 2017) and distilled white vinegar (Kilonzo-Nthenge and Liu., 2019) against different microorganisms (*E. coli, S. aureus, Flavobacterium psychrophilum, Aspergillus niger, Penicillium digitatum, Streptococcus pyogenes, Klebsiella oxytoca, Enterococcus faecalis, Bacillus cereus* etc.).

Furthermore, some researchers utilized antibiotics in their studies to compare the results obtained from vinegar samples. Yang *et al.* (2016) studied by disc inhibition zone on antimicrobial activity of wood vinegar against *E. coli, Acinetobacter baumannii S. aureus, Pseudomonas aeruginosa* and compared its effect with tetracycline as an antibiotic. It was reported that tetracycline displayed greater disc zones against *E. coli* and *P. aeruginosa*. In another study, the antimicrobial effectiveness of apple cider vinegar was tested on *Staphylococcus aureus, E. coli, Salmonella paratyphi* A, *Salmonella paratyphi* B and it was compared with the effect of ciprofloxacin. Vinegar samples exhibited higher antimicrobial activity than antibiotics against all tested microorganisms except *S. paratyphi* A (Saqib, 2017). Besides, Choi *et al.* (2015) carried out a study to determine the antimicrobial activity of fermented dark vinegar (FVD), which is a traditional Japanese product obtained by fermentation of unpolished rice. They compared the antimicrobial activity of vinegar with two antibiotics (Carbenicillin, Tetracycline) and propionic acid against various bacteria species. As a result, 3 years of matured FVD had greater antimicrobial activity on all microorganisms than the utilized antibiotics. The research emphasized that the antimicrobial properties of vinegar changed depending on its pH, temperature, acetic acid content, ionic strength (Samad *et al.*, 2016) as well as production method and the initial number of test

microorganisms. Although some research was available on the antimicrobial effects of vinegar, there is limited information about the antimicrobial capacity of traditional vinegar.

This research aims to determine the antimicrobial activity of different kinds of vinegar produced with the traditional method on some important foodborne pathogens by using the antimicrobial disc diffusion method. Besides, the organic acid content, antioxidant capacity and phenolic component content of various kinds of vinegar were examined and their relationship with the antimicrobial effect was examined.

Materials and methods

Production of vinegar

The fruits used in vinegar production were obtained from the local market. The apple cider (AV), pomegranate (PV), grape (GV), sour cherry (SV), hawthorn vinegar (HV) were produced according to the traditional vinegar production technique used by Budak (2010). A flow chart of the process is presented in Figure 1. Production consisted of two fermentation stages: ethanol fermentation (30 days) and acetic acidic fermentation (60 days).

Evaluation of the antibacterial effect

Antibacterial effects of vinegar samples were determined using the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (American Society for Microbiology, 2016). Cultures of *E. coli ATCC 26922, E. fecalis ATCC 29212, S. aureus ATCC 25923*, and *C. jejuni ATCC 17028* were obtained from the culture collection of Suleyman Demirel University, Food Engineering Department. Stock cultures of bacteria were grown in nutrient broth at 37°C for 24 h before use. All selected strains of bacteria were adjusted to 0.5 McFarland's turbidity standard (10^{6-7} CFU/ml) using sterile nutrient broth and after vortexing of each organism used in experiments.

100µL of all bacterial suspensions was spread on the Petri dishes including Mueller-Hinton Agar (Merck). The antimicrobial susceptibility test discs (Bioanalyse, Great Britain) were impregnated with 20µl of the vinegar samples and placed on the inoculated Mueller-Hinton Agar (MHA). Negative control (4% acetic acid solutions) was also impregnated on discs and placed on the agar. Different antibiotic discs were used as positive controls according to the sensitivity of each bacterial species. The antibiotics used were penicillin (10IU) (P) and vancomycin (5µg) (V) for *S. aureus*; ampicillin (10µg) (A1) and imipenem (10µg) (I) for *E. coli*; ampicillin (2µg) (A2) and vancomycin (5µg) (V) for *E. fecalis*; erythromycin (15µg) (E) and ciprofloxacin (5µg) (C) for *C. jejuni*. Then, the Petri plates were incubated at 37°C for 24 h. The antimicrobial activity of samples was evaluated by measuring zones of inhibition surrounding tested bacteria.

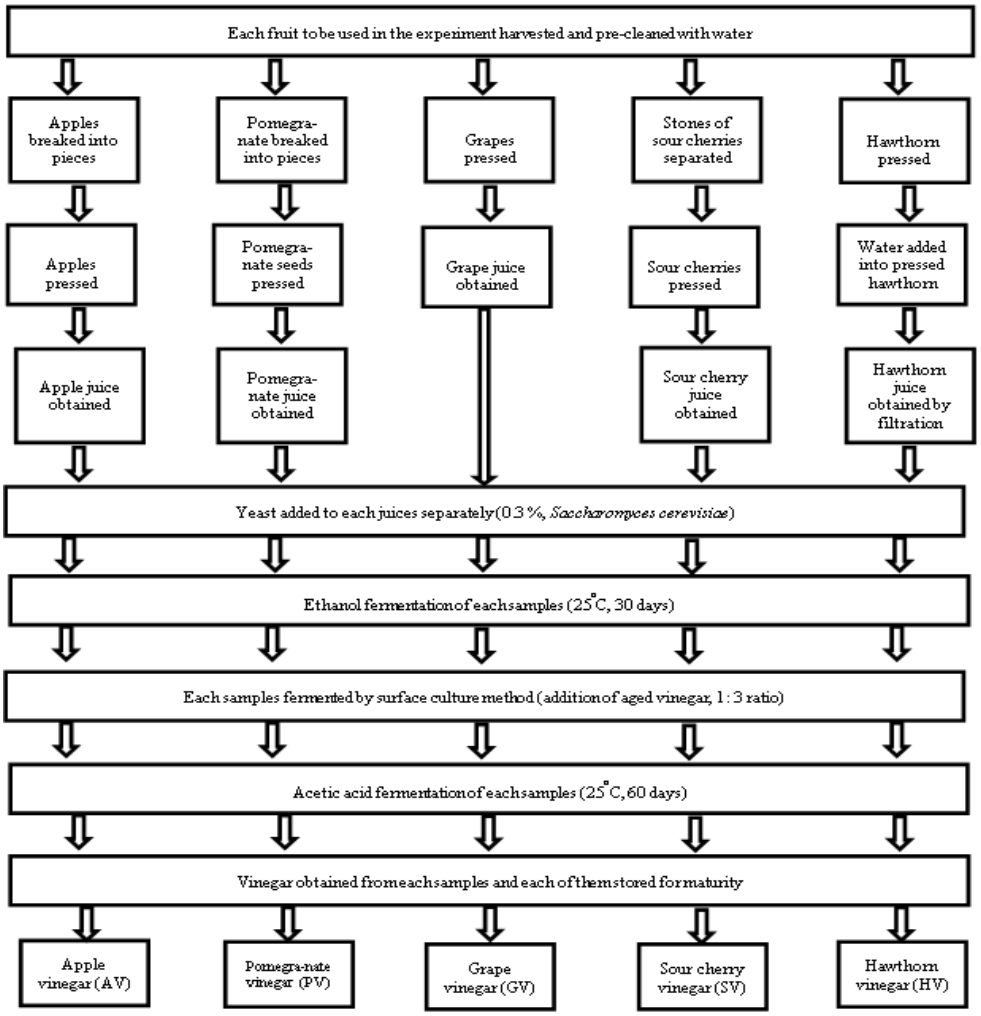


Figure 1. A flow chart of the process for the obtainment of vinegar.

Titratable acidity

The total titratable acidity was determined according to AOAC (2000).

Total phenolic content and antioxidant activity analysis

The total phenolic contents of the samples were determined spectrophotometrically (Shimadzu Scientific Instruments, Inc., Tokyo, Japan) according to the Folin-Ciocalteu method (Singleton *et al.*, 1999). The measurement was expressed as milligrams of gallic acid equivalents (GAE) L^{-1}.

The antioxidant activity of the samples was measured using the Oxygen Radical Absorbance Capacity (ORAC) and 2,2′-azinobis (3-ethlybenzothiazoline)-6-sulfonic acid (ABTS) methods. The ORAC assay was carried out spectrofluorometrically using a Synergy™ HT Multi-Detection Microplate Reader

(Winooski, Vermont, USA) by kinetic measurement (Huang *et al.*, 2002; Davalos *et al.*, 2005). The reading was performed at an excitation-emission wavelength of 485 to 520 nm using KC4™ Data Reduction Software (BioTek Instruments, Winooski, VT) (Prior and Cao, 2000). The ABTS ($TEAC^{-}$) assay was carried out spectrophotometrically. Absorbance was measured at a wavelength of 734 nm. Results for both analyses were expressed in micromoles of Trolox equivalent (TE) per milliliter.

Phenolic compound analysis

Phenolic compounds were determined by reversed-phase, high-performance liquid chromatography (HPLC) equipment (Shimadzu Scientific Instruments, Kyoto, Japan) with a diode array detector (Shimadzu SPD-M20A) at 198 nm. C18 column (Gemini C18, 150 × 3 mm, 5 µm, 110A, Phenomenex, CA, USA) was used. The mobile phase consisted of 18% ACN and 50mM o-phosphoric acid (pH 4.5) at a 0.8 ml/min flow rate with isocratic elution. The column oven temperature was at 30°C. The samples were diluted 10 times and passed through an ana 0.45 µm polytetrafluoroethylene (PTFE) filter and injected to the system (20 µl). Gallic acid, chlorogenic acid, catechin, epicatechin, caffeic acid, ellagic acid, p-coumaric acid and ferulic acid (Sigma Chemical Co., Bornem, Belgium) were used as external standards to establish the calibration curves. Identification and quantitative analysis were conducted by comparison with standards.

Organic acid analysis

Organic acid was analyzed by HPLC (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan) and determined as the amount and component of organic acid. Inertsil ODS-3V C18 (GL Sciences Inc.) (250x4.60 mm, 5 µm) was utilized as a column. The temperature of the column oven was adjusted to 30°C Mobile phase was 50 mM HCl solution at a flow rate of 1.0 mL/min. Samples were passed through a 0.45 µm polytetrafluoroethylene filter (Membran Solutions) and injected as 20 µL into the system. DAD detector 210 nm wavelength was utilized.

Statistical analysis

The statistical calculations were by One-Way Analysis of Variance (ANOVA) test using IBM SPSS v. 22.0 (SPSS Inc., Chicago, USA). To assess the significantly different results ($p < 0.05$) between the vinegar samples, Tukey's test was used.

Results and discussion

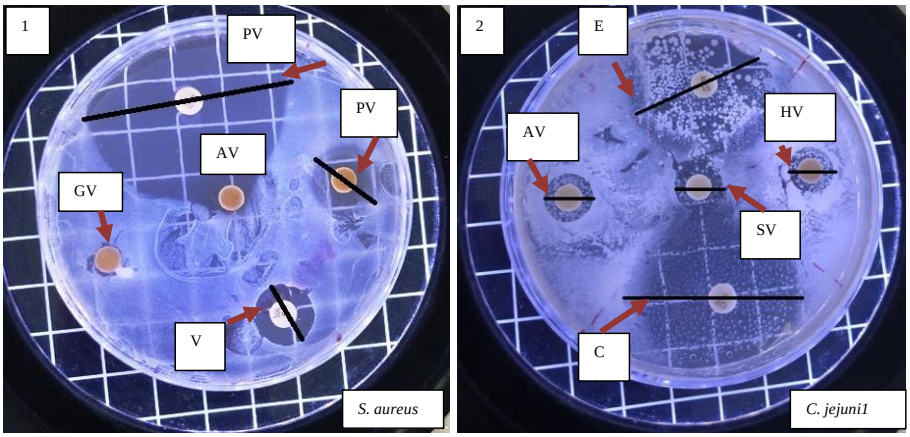
Antimicrobial activity of vinegar

The disc diffusion assay is commonly used to determine the sensitivity of pathogenic bacteria to various antimicrobial materials. In this study, the antibacterial activity of different vinegar samples (apple cider, pomegranate, grape, sour cherry, hawthorn) against Gram-positive bacteria (*E. fecalis* and *S. aureus)* and Gram-negative bacteria (*E. coli* and *C. jejuni*) by Kirby-Bauer Disk Diffusion method. The maximum inhibition zone diameters exhibiting the antibacterial effect of different kinds of vinegar are given in Table 1. Figure 2 shows the experimental results.

Two different special antibiotic discs (AD) were used as controls for each microorganism according to known bacterial susceptibility patterns (Wackett, 2013).

Among the vinegar samples, only pomegranate vinegar was effective on *Staphylococcus aureus*. In a similar study, it was found that pomegranate vinegar showed higher antibacterial activity on *S. aureus* than on *E. coli* and *S. typhimirium* (Bakir *et al.*, 2017). The zone diameter of the pomegranate vinegar against *S. aureus* was greater than vancomycin while the maximum inhibition zone diameters of other kinds of vinegar showed no activity against *S. aureus*. The maximum inhibition zone diameter was observed as pomegranate vinegar while the minimum inhibition zone diameter was determined as grape vinegar against *E. fecalis.* The inhibition zones of vancomycin and pomegranate vinegar were similar to each other. The maximum inhibition zone diameter was determined as PV, AV, HV, while the effect of GV has not been observed against *E. coli.* PV, AV and HV samples have been observed to be as effective as ampicillin against *E. coli.* The AV vinegar sample has been observed as the maximum inhibition zone diameter against *C. jejuni.*

The maximum inhibition zone diameter of pomegranate vinegar was determined 15 mm for *E. coli* and 9 mm for *C. jejuni* as representing Gram-negative bacteria while 15 mm for *S. aureus* and 14 mm for *E. fecalis* as representatives of Gram-positive bacteria (Table 1). It was shown that zone images of different vinegar and antibiotics exhibited antibacterial effects of tested agents on microbial growth after incubation at 37°C for 24h in Figure 2. The highest zone diameter of apple cider vinegar was observed at 13 mm against *E. fecalis* as representatives of Gram-positive bacteria while 16 mm and 19 mm against *E. coli* and *C. jejuni*, respectively as representatives of Gram-positive bacteria (Table 1 and Figure 2).



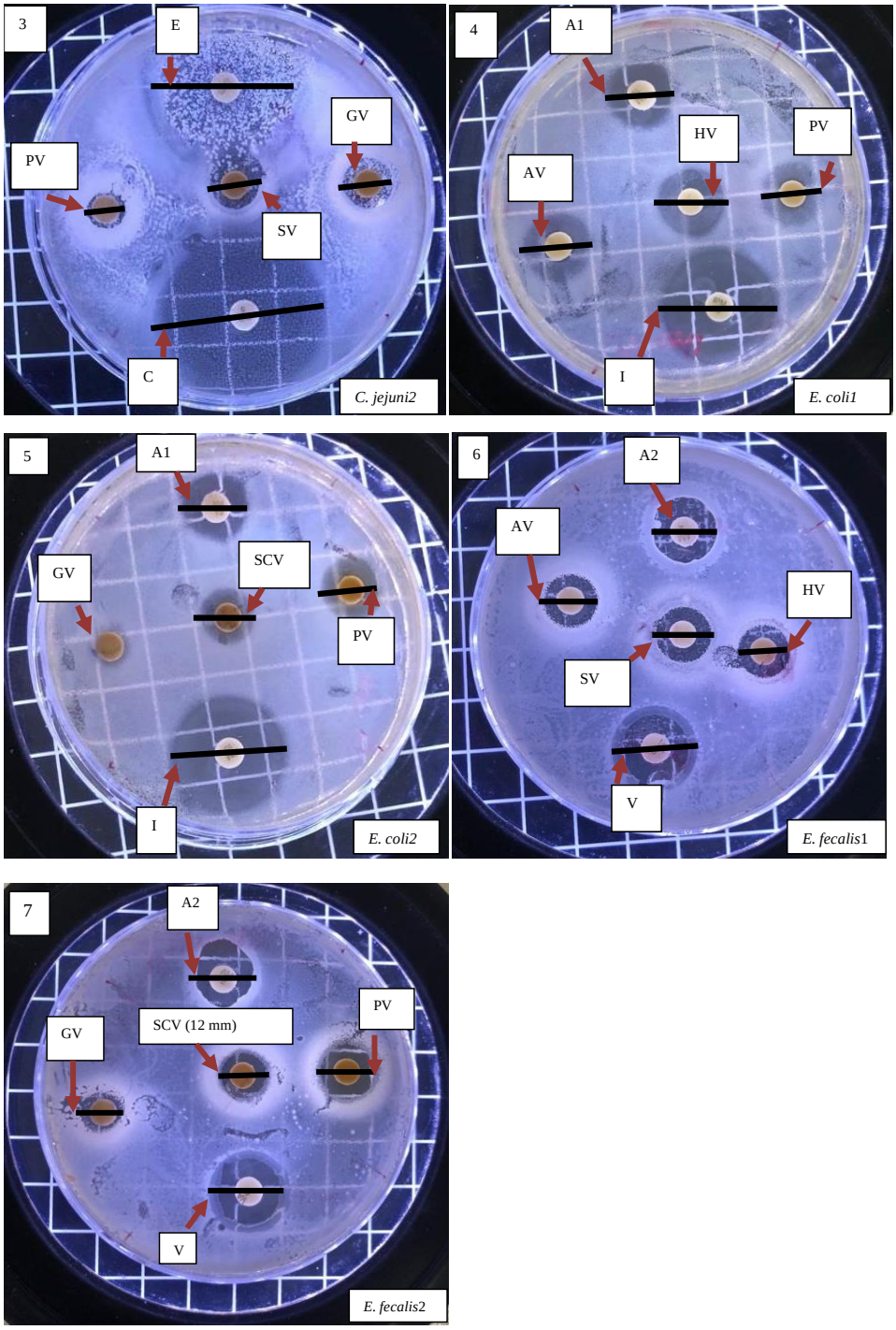


Figure 2 Zone images of varying vinegar and antibiotics showing antibacterial effects of tested agents on microbial growth after incubation at 37 °C for 24h. 1.) *S. aureus*; 2.) *C. jejuni*1, 3.) *C. jejuni*2, 4.) *E-coli1*, 5.) *E-coli2*, *6.) E. fecalis*1 *7.) E. fecalis*2.

Table 1. The maximum inhibition zone diameters (mm) for antibacterial susceptibility of different kinds of vinegar.

	AV	PV	GV	SV	HV	AD 1	AD 2
S. aureus	-	18	-	-	--	**Penicillin** 48	**Vancomycin** 15
E. faecalis	13	14	9	12	13	**Ampicillin** 18	**Vancomycin** 15
E. coli	16	15	-	13	15	**Ampicillin** 16	**Imipenem** 26
C. jejuni	19	9	11	12	13	**Erythromycin** 29	**Ciprofloxacin** 35

AV Apple Vinegar, PV Pomegranate Vinegar, GV Grape Vinegar, SV Sour Cherry Vinegar, HV Hawthorn Vinegar, AD Antibiotic Disc.

Single and combined antimicrobial impacts of mustard flour and acetic acid were determined in different concentrations against *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium strains. The individual acetic acid application was detected as the most effective treatment against *E. coli* and *L. monocytogenes* (Rhee *et al.*, 2003).

The inhibition zone of traditional apple vinegar was tested on four clinical isolates. It was observed *Enterobacter kobei* (13 mm), *Enterobacter cloacae* (14 mm), *S. aureus* (11,33 mm), *E. coli* (12,66 mm) and antimicrobial drug ceftriaxone were used as a control (12 mm) (Kalaba *et al.*, 2019). In this study, the apple vinegar sample had a greater inhibition zone value for *E. coli* (16 mm), while it had a lower inhibition zone value for *S. aureus* (0.0 mm).

Duru (2016) carried out a study on the antimicrobial effectiveness of traditionally produced grape, apple and pomegranate vinegar on *F. psychrophilum*. Grape vinegar had the greatest antimicrobial activity (55 mm) on *F. psychrophilum*, and then pomegranate vinegar (54 mm) and apple vinegar (50 mm). Even our highest value for the inhibition zone value (19 mm against *C. jejuni* from apple vinegar application) was lower than the results of these studies.

Gopal *et al.* (2017) reported that several concentrations (1, 10, 25, 50, 100%) of apple cider vinegar (ACV) were applied to *Candida albicans, Saccharomyces cerevisiae* and *A. niger* to test the antimicrobial activity of the vinegar. As a result, 100% ACV exhibited antimicrobial activity on *C. albicans* (5 mm), while 25% ACV exhibited an antimicrobial effect on *S. cerevisiae* (3 mm). On the other hand, 100% ACV demonstrated antimicrobial activity on *A. niger* (25 mm) (Gopal *et al.*, 2017). Also, similar to the results of these studies, Ousaaid *et al.* (2021) reported that traditional ACV had an antimicrobial effect on tested microorganisms such as *Vibrio cholerae*, *Candida tropicalis*, *C. Albicans*, *E. coli O157:H7* and *S. typhi*. According to Ousaaid *et al.* (2021), the antimicrobial properties of ACV are related to its bioactive compounds like organic acids and phenolic compounds which are similar to our findings and results.

Choi *et al.* (2015) utilized the paper disc diffusion method to test the antimicrobial susceptibility of various microorganisms against fermented dark vinegar (FDV) produced from unpolished rice. It was reported that the same antimicrobial activity of 1 and 3-year matured-FDV on *S. aureus* (15 mm), while this was greater than other tested antimicrobials such as Carbenicillin (50 µg/ml) and Tetracycline (50 µg/ml). Also, 1 and 3-year matured-FVD exhibited antimicrobial activity on *E. coli* (13 mm), which was lower than the impact of propionic acid, but greater than Carbenicillin (50 µg/ml) (Choi *et al.*, 2015). Our vinegar samples had equal (sour cherry vinegar) or greater antimicrobial effect on *E. coli*, except grape vinegar (0.0 mm). For *S. aureus*, our pomegranate vinegar sample (18 mm) had a greater inhibition zone, while our other vinegar samples had no inhibition zone (0.0 mm) for *S. aureus*.

Wood vinegar was investigated for its inhibitory properties against *E. coli, A. baumannii, S. aureus, P. aeruginosa* and Ornithine-resistant *S. aureus*. It was represented that the vinegar had the greatest inhibitory effect on *S. aureus* (19.00 ± 1.00 mm). Also, it had an antimicrobial effect on *E. coli* (15.20 ± 0.40 mm) (Yang *et al.*, 2016). Wood vinegar had a greater antimicrobial effect on *S. aureus* than our pomegranate vinegar sample (18 mm). However, for *E. coli*, our vinegar samples, except grape vinegar, had a greater or equal antimicrobial effect. In another study (Zhang *et al.*, 2019), wood vinegar produced from pyrolysis of polyploidy mulberry branches was tested against *E. coli, B. cereus, B. subtilis, A. aerogenes*, and *S. aureus* to investigate its antimicrobial activity. Wood vinegar samples exhibited antibacterial activities between 13.5-25.5 mm diameter of inhibition among all tested bacteria. A recent study (Desvita et al. 2022) investigated that wood vinegar produced from cocoa pod shells exhibited antimicrobial effectiveness against *Candida albicans* and *Aspergillus niger* measured as 6-6.12 mm and 6-6.14 mm inhibitory diameter zones, respectively. Another recent study (Xue *et al.*, 2022) examined the impact of various rafination techniques on the quality of the product. For this purpose, six different pathogenic bacteria were tested with wood vinegar samples. Wood vinegar exhibited antimicrobial activity with various ratios as inhibition rates between 14.29-85.71%.

The antimicrobial activity of grape vinegar and apple vinegar was investigated against *S. aureus, E. coli* and *Pseudomonas aeroginosa*. The average inhibition zone diameter of *S. aureus* was determined 9.67-13.33 mm for grape vinegar and 9.00-13.33 mm for apple vinegar. On the other hand, the average inhibition zone diameter of *E. coli* was 9.33-14.67 mm for grape vinegar and 10.17-14.00 mm for apple vinegar (Kelebek *et al.*, 2017). Our grape vinegar had no inhibitory effect on *S. aureus* and *E. coli*. However, our apple vinegar had greater (16 mm) inhibitor activity for *E. coli*, but no activity for *S. aureus*.

Mulberry vinegar was tested for its antimicrobial activity against nine different microorganisms. *S. aureus* had the greatest antimicrobial effect (28 mm), while *E. coli* had the lowest antimicrobial effect (5.3 mm). Also, *E. faecalis* had a relatively high antimicrobial effect (19.6 mm) (Aydın, 2013). In our study, pomegranate vinegar samples had lower antimicrobial activity (18 mm) against *S. aureus*.

However, all of our vinegar samples exhibited greater antimicrobial activity on *E. coli*, except grape vinegar.

Hawthorn vinegar produced traditionally from hawthorn fruit was tested for its antimicrobial activity on some gram positive and negative microorganisms. As a result of this study, from the highest antimicrobial activity of hawthorn vinegar to the lowest one was ranked as *C. jejuni*, *L. monocytogenes*, *E. coli*, *S. aureus* and *E. faecalis*, respectively (Özdemir *et al*., 2021).

Titration Acidity

The titration acidity values of the kinds of vinegar were in the range of 4.42-4.75%. According to TS 1880, the total acid content of wine vinegar should be at least 4g/100mL in terms of acetic acid in Turkey (Anonymous, 2003). Our results were in conformity with Turkish Standards.

Antioxidant Properties

Total phenolic contents (TPC) and values of antioxidant capacity (ORAC and $ABTS^+$) of vinegar samples are represented in Table 2. PV had significantly higher ORAC value with 54.21 mmol TE/L ($p < 0.05$) and SV had the highest values of ABTS and TPC with values of 27.35 mmol TE/L and 3511.7 mg GAE/L, respectively ($p < 0.05$).

Table 2. Antioxidant capacity of vinegar samples.

Sample	Titration acidity (%)	ORAC (mmol TE/L)	$ABTS^+$ (mmol TE/L)	TPC (mg GAE/L)
AV	4.42 ± 0.01^{e}	17.57 ± 1.22^{c}	10.27 ± 1.20^{c}	948.595 ± 24.54^{a}
PV	4.75 ± 0.02^{a}	54.21 ± 0.48^{a}	22.33 ± 1.48^{ab}	2854.1 ± 42.86^{b}
GV	4.55 ± 0.01^{c}	30.60 ± 2.56^{bc}	17.54 ± 2.45^{b}	1583.66 ± 28.42^{c}
SV	4.62 ± 0.02^{bc}	46.478 ± 4.74^{ab}	27.35 ± 1.46^{abc}	3511.7 ± 38.64^{d}
HV	4.53 ± 0.03^{cd}	23.52 ± 12.72^{a}	23.01 ± 0.70^{ab}	2420.73 ± 22.7^{e}

Data expressed as mean±standart deviation. Different lowercase letters in the same column indicate significant differences between the samples ($p < 0.05$). n.d.: not detected.

Budak (2015) reported that TEAC and TPC values of pomegranate vinegar were between 16-20 µmol/ml and 1200-2200 mg GAE/L, respectively. Özen *et al.* (2020) stated that antioxidant values of sour cherry vinegar according to a TEAC assay were 31.39 mmol TE mL^{-1}. In another study, the commercially processed sour cherry and pomegranate vinegar exhibited the highest antioxidant levels which were following our results (Ozturk *et al*., 2015). Pomegranate (*Punica granatum*) has high antioxidant activity. This is attributed to its anthocyanins content such as delphinidin, cyanidine and pelargonidine, and ellagitannins (Budak, 2015). Sour cherry has rich bioactive content (e.g. cyanidin, 3-rutinoside, peonidin, 3-glucoside, isorhamnetin, quercetin, ferulic acid, chlorogenic acid, p-coumaric acid) (Kirakosyan *et al*., 2009; Jakobek *et al*., 2009).

In the vinegar samples, the quantification of individual phenolics was performed by HPLC. The gallic acid, chlorogenic acid, p-coumaric acid, caffeic acid, ferulic acid, ellagic acid, catechin and epicatechin were identified as phenolic compounds in vinegar samples (Table 3). Similar to TPC and ABTS results, gallic acid had its highest concentration in SV. Ellagic acid was a constituent of only PV as the major component value with 133.04 mg/L. Catechin was the highest concentration in the PV sample. PV had a higher ORAC value than other samples, it was thought to be related to high catechin content. Meyer *et al*. (1998) reported that catechin had the highest antioxidant activity than caffeic acid, cyanidin, quercetin and ellagic acid.

Table 3. Phenolic compounds of vinegar samples (data expressed as ppm).

	AV	PV	GV	SV	HV
Ga	50.24±0.45^{cde}	25.25±4.18^{de}	18.23±4.67^{e}	183.12±24.56^{a}	76.89±3.65^{bc}
Chl	68.66±2.77^{a}	60.35±6.38^{a}	17.02±0.17^{b}	67.04±2.48^{a}	53.83±4.85^{a}
Cat	8.22±0.15^{cd}	80.9±1.84^{a}	27.50±3.20^{b}	10.05±0.54^{c}	4.65±0.12^{ce}
Ec	17.00±0.32^{a}	10.5±1.50^{bc}	8.20±1.20^{c}	20.14±2.45^{a}	3.37±1.16^{cd}
CfA	10.02±0.15^{bd}	n.d.	13.63±3.84^{bc}	14.30±3.86^{b}	37.37±2.80^{a}
Ea	n.d.	133.24±22.46	n.d.	n.d.	n.d.
p-coum	7.26±0.01^{c}	n.d.	23.22±0.23^{a}	7.48±1.48^{bc}	n.d.
Fa	n.d.	n.d.	0.35±0.27^{a}	5.11±0.36^{b}	n.d.

Data expressed as mean±standart deviation. Different lowercase letters in the same row indicate significant differences between the samples ($p < 0.05$). n.d.: not detected. Ga: Gallic acid, Chl: Chlorogenic acid, Cat: Catechin, Ec: Epicatechin, CfA: Caffeic acid, Ea: Ellagic acid, p-Coum: P-coumaric acid, Fa: Ferulik acid

Organic acid content

Organic acids in vinegar are formed during fermentation by the biochemical and metabolic activities of microorganisms. Especially, acetic acid bacteria may produce many different kinds of organic acids such as acetic, tartaric, lactic, malic, and citric acids as a conclusion of the oxidation of sugars and alcohols. However, acetic acid is the major dominant acid among the organic acid content of the vinegar. According to various studies, acetic acid is effective against *Bacillus* spp., *Clostridium* spp., *L. monocytogenes*, *Salmonella*, *S. aureus*, *E. coli*, *C. jejuni* and *Pseudomonas*. Organic acid types and contents affect the sensory and functional properties of the product. The antimicrobial activity of various kinds of vinegar is mostly associated with organic acid content. The organic acids of the vinegar pass into the cell membranes of microbial cells causing the death of cells. The antimicrobial activity of organic acids mainly depends on the strain of bacteria, environmental temperature, pH and acid concentrations as well as ionic strength. In various studies, antimicrobial activities and organic acids have been determined against different microorganisms (Skřivanová and Marounek, 2007; Budak *et al*., 2014). The results showed that different vinegar samples contained various amounts of organic acids (Figure 3 and Table 4). The contents of oxalic, malic, citric, acetic, lactic and succinic acid were determined as the organic acids of vinegar. Acetic acid was the major organic acid and the acetic acid content of

samples ranged between 28.977-48473 mg L^{-1}. In fruit vinegar, the sugar turns into alcohol with the effect of yeasts followed by alcohol is converted to acetic acid by the action of the bacteria (Shahidi et al., 2008). For this reason, the sugar ratio in fruits is an important factor to form specifically acetic acid and other kinds of organic acids. GV has the highest acetic acid content as the grape is a fruit with high sugar content. Grape juice has higher sugar content rather than other fruit samples (Cemeroğlu, 2013).

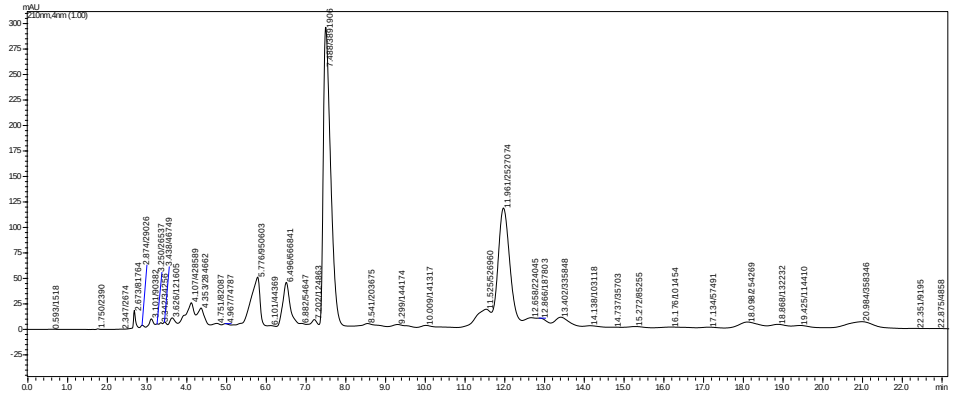


Figure 3. Organic acid chromotograms of apple vinegar as an example.

Table 4. Organic acids of vinegar samples (data expressed as ppm).

	AV	PV	GV	SV	HV
TA	1265.00±22.05^{a}	n.d.	2011.00±12.4^{b}	2580.00±40.00^{c}	n.d.
OA	269.00±1.20^{d}	354.00±1.40^{c}	1083.00±11.00^{a}	267.00±1.20^{e}	386.00±40.00^{bc}
CA	98.00±1.40^{e}	180.00±1.60^{d}	280.00±1.30^{c}	1537.00±19.00^{a}	808.00±26.88^{b}
MA	4210.66±12.54^{a}	378.09±625.00^{bd}	665.08±4.87^{c}	265.31±2.50^{d}	630.27±4.20^{ec}
AA	47474.00±87.00^{a}	39028.00±94.24^{b}	48180.00±74.82^{c}	28977.00±47.00^{d}	39650.00±35.00^{e}
LA	120.55±2.06^{d}	n.d.	420.62±2.55^{c}	4387.64±42.32^{b}	6546.79±36.91^{a}
SA	256.00±2.10^{a}	n.d.	324.00±2.00^{b}	n.d.	n.d.

Data expressed as mean±standart deviation. Different lowercase letters in the same row indicate significant differences between the samples ($p < 0.05$). n.d.: not detected. TA: Tartaric acid, OA: Oxalic acid, CA: citric acid, MA: malic acid, AA: acetic acid, LA: lactic acid, SA: Sucsinic acid.

In general, PV had moderate organic acid and phenolic contents. However, in the sum, it had significantly higher ORAC and TPC values ($p < 0.05$). In favour of this, PV showed the highest antimicrobial activity against test microorganisms among the vinegar samples. This is probably because phenolic compounds and organic acids promote together antimicrobial activity as mentioned by Kahraman *et al*. (2022).

Conclusions

Traditional vinegar is one of the natural food ingredients and food preservatives that have been emphasized by consumers and researchers in recent years due to its

bioactive compounds and health effects. While the protective properties of vinegar are mainly associated with a high organic acid content, it should be taken into account that phenolic compounds may also contribute to antimicrobial properties. Overall, the vinegar with the highest antimicrobial activity was detected as pomegranate vinegar against the gram-positive microorganism group and apple vinegar against the gram-negative microorganism group in this research. According to the results, it can be concluded that the raw material has a decisive role in the antibacterial properties and bioactive content of vinegar. When the results obtained in this study are examined, it can be concluded that the antioxidant, phenolic and organic acid content is effective on the antimicrobial effect of vinegar.

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