RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2024; 38 (3): 211 - 218
http://www.fusabil.org

Identification of Bioactive Compounds in Various Vinegars and Determination of Their Antimicrobial Properties Against Foodborne Microorganisms

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The aim of this study was to determine the bioactive compounds in a variety of vinegars (date, fig, mulberry, black mulberry, and pomegranate) and to test their *in vitro* antimicrobial activity against major foodborne pathogenic bacteria. Organic acids, volatiles, individual phenolic and flavonoid compounds, total phenolic content (TPC) and pH values were identified in vinegars. In addition, minimum inhibitory concentration (MIC), minimum bactericidal and fungicidal concentration (MBC and MFC) and diameter of inhibition zones of vinegars were determined against *Escherichia coli* O157:H7, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Staphylococcus aureus* and *Aspergillus parasiticus*. A total of five different organic acids, 33 to 51 volatiles, and 13 to 21 polyphenols were identified in the vinegars. The pH of the vinegars were found to range between 3.27 and 3.74, and the TPC values were determined to be between 96.74 and 982.61 mg GAE/L. The MIC, MBC, and diameter of inhibiton zones against the tested strains were determined to be between 1.56% and 20.83%, 6.25% and 50%, and 8.22 and 19.81 mm, respectively. The results of this study indicate that the vinegars have a diverse array of bioactive compounds at varying concentrations, which consequently displayed a range of antimicrobial activity against the tested strains. The black mulberry vinegar exhibited the highest antimicrobial activity compared to the other vinegars. In conclusion, vinegar can be employed as a natural preservative to enhance the microbiological quality and safety of foods.

Key Words: *Vinegar, characterization, antimicrobial activity, foodborne microorganism*

Çeşitli Sirkelerdeki Biyoaktif Bileşiklerin Tanımlanması ve Gıda Kaynaklı Mikroorganizmalara Karşı Antimikrobiyal Özelliklerinin Belirlenmesi

Bu çalışmada, hurma, incir, dut, karadut ve nar sirkelerindeki biyoaktif bileşenlerin tespiti ve majör gıda kaynaklı patojen bakterilere karşı in- vitro antimikrobiyal aktivitelerinin test edilmesi amaçlanmıştır. Bu amaçla, organik asit, uçucu bileşen, bireysel fenolik ve flavonoid bileşikler, toplam fenolik içerik (TFİ) ve pH analizleri yapılmıştır. Ayrıca, *Escherichia coli* O157:H7 ATCC 43895, *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 13932, *Salmonella* Typhimurium ATCC 14028, *Salmonella* Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC 25923 ve *Aspergillus parasiticus* NRRL 2999 patojenlerine karşı Minimum İnhibitör Konsantrasyon (MİK), Minimum Bakterisidal ve Fungusidal Konsantrasyon (MBK ve MFK) ve inhibisyon zonları tespit edilmiştir. Yapılan analizler sonucunda sirke çeşidine göre değişmekle birlikte, beş farklı organik asit, 33 ile 51 arasında uçucu bileşik, 13 ile 21 arasında bireysel fenolik ve flavonoid bileşen belirlenmiştir. Sirke örneklerinin pH değerleri 3.27 - 3.74, toplam fenolik içeriklerinin ise 96.74- 982.61 mg GAE/L arasında olduğu kaydedilmiştir. Test patojenlerine karşı MİK, MBK, MFK ve inhibisyon zon değerlerinin sırasıyla %1.56 - 20.83, %6.25 - 50 ve 8.22 - 19.81 mm arasında olduğu ölçülmüştür. Bu çalışmanın sonuçları, sirkelerin biyoaktif madde içerikleri bakımından farklılık gösterebildiği ve bundan dolayı antimikrobiyal aktivitenin de test edilen mikroorganizmalara karşı değişkenlik gösterebileceğini ortaya koymuştur. Sonuç olarak sirke, gıda maddelerinin mikrobiyolojik kalitesini ve güvenliğini arttırmak için doğal bir koruyucu olarak kullanılabilir.

Anahtar Kelimeler: *Sirke, karakterizasyon, antimikrobiyal aktivite, gıda kaynaklı mikroorganizma*

Received : 20.07.2024
Accepted : 14.08.2024

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Introduction

The global demand for a healthy lifestyle has led to a continuous search for novel functional foods. In this context, fermented foods have been gaining interest in the daily diet. Vinegar is one of the oldest fermented foods with a history spanning centuries. It is recognized as a safe and natural food that contains no additional ingredients. In addition, vinegar is generally recognized as safe (GRAS). In recent decades, vinegar has been used as a nutritional supplement to prevent a variety of infectious diseases, including severe acute respiratory syndrome (SARS). It has also become a substance used as a disinfectant (1-6).

Vinegar is produced by bacteria and yeast metabolism through the fermentation of carbohydrates (7). A wide range of raw materials, including cereals (rice, barley, and millet etc.), fruits (apples, grapes, and coconuts etc.), vegetables (onion etc.), animal derived foods (honey and whey), sugar cane and roots can be used to produce vinegar (3, 8). The production of traditional vinegar involves a two-stage fermentation process.

In the initial stage, carbohydrates are converted to alcohols by the metabolic actions of yeass, particularly those belonging to the *Saccharomyces* species. Subsequently, the alcoholic compounds are degraded to carboxylic acids, including acetic acid. The second stage of fermentation is facilitated by the involvement of various bacteria belonging to the genera of *Acetobacter, Gluconobacter,* and *Gluconacetobacter* (9).

Due to producing through the fermentation carbonhydrates, vinegars contain a variety of bioactive compounds, including organic acids, polyphenols, and volatiles (7). However, the amount and variety of bioactive compounds may vary depending on the raw material and the fermentation process, including temperature, duration, and microorganisms. (6, 10). It is well documented that vinegars exhibit a variety of bioactive properties. In this manner, a number of bioactive properties have been identified in vinegars, including antimicrobial, anti-inflammatory, antidiabetic, anticarcinogenic, antioxidative, kidney stone-prevention, blood sugar-lowering, and immune-stimulating effects (3, 10-12). Additionally, vinegar has been shown to promote digestion and facilitate weight loss (1, 3, 13-16).

The contamination of foods by pathogenic microorganisms (mainly bacteria) may result in the occurrence of foodborne diseases and outbreaks. In this context, the European Food Safety Authority (EFSA, 2022) have reported that the most prevalent foodborne pathogens include *Salmonella* spp., Shiga-toxigenic *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (17). Furthermore, certain fungal strains, including *Aspergillus* spp., are not only pathogenic to humans by producing mycotoxins, but also contribute to the deterioration of a great variety of foods (18). Consequently, further investigation is still required to identify effective decontamination approaches and/or preservatives that can be employed to prevent and control these foodborne zoonoses and spoilage microorganisms. (2, 8, 19).

As previously stated, numerous studies have demonstrated the presence of a diverse array of bioactive compounds and beneficial properties commonly associated with vinegars. However, the complexity of the raw materials and the extensive range of fermenting microorganisms suggest that there is a significant scope for further investigation into the bioactive potential of vinegars (16). Therefore, further characterization and testing the antimicrobial activity of vinegars produced from different raw materials are still required. In the light of this aforementioned information, the aim of the current research is to identify the bioactive compoounds of vinegars derived from different raw materials, including date, fig, mulberry, black mulberry, and pomegranate. Additionally, it was aimed to evaluate the antimicrobial activity of these vinegars against foodborne microorganisms, including *Escherichia coli* O157:H7, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Staphylococcus aureus*, and *Aspergillus parasiticus*.

Materials and Methods

Materials: The organic date, fig, mulberry, black mulberry, and pomegranate vinegars was obtained from a local market in Elazığ, Turkey.

Methods

Characterization of Vinegars: The organic acids, volatiles, pH, TPC, individual phenolic and flavonoid compounds of the vinegars were determined by the method outlined in our previously published research (8). The organic acids were determined by using a Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC, Shimadzu LC 20AD, Japan). The identification of volatiles was conducted through the using of Gas Chromatography-Mass Spectrometry. For the determination of TPC, Folin-Ciocalteu's assay was used, and the results were expressed as mg gallic acid equivalent GAE/L. Individual phenolic compounds in the vinegars were determined by HPLC (Shimadzu LC 20AD Corporation, Kyoto, Japan), and the results are given as mg/L.

Determination of *in vitro* Antimicrobial Activities of Vinegars: The *in vitro* antimicrobial activity of the vinegars were evaluated against *Escherichia coli* O157:H7 ATCC 43895, *Escherichia coli* ATCC 35218, *Listeria monocytogenes* ATCC 13932, *Salmonella* Typhimurium ATCC 14028, *Salmonella* Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC 25923, and *Aspergillus parasiticus* NRRL 2999. The diameter of inhibition zones, MIC, MBC, MFC values of the vinegars were determined against tested strains. Prior to conducting in vitro antimicrobial tests, the vinegar samples were filtered through a 0.22 µm pore size membrane to remove the initial microbial load. The disc diffusion method was used to determination of the diamater of inhibition zones. For this purpose, the tested microorganisms were separately inoculated onto Mueller-Hinton agar at a level of 6.0 $\log_{10}$. Following inoculation, the plates were incubated at ambient temperature for 10 minutes to allow for microbial attachment to the agar surface. Subsequently, 20 µL of the filtered vinegar samples were embedded in sterile empty filter papers, which were then placed on the plates. The plates were then incubated at 37 °C for 24 h. Afterwards, the diamater of inhibition zones were measured with digital callipers (20). The MIC values of the vinegars were determined using the broth dilution method. The two-fold dilutions starting from 50% concentration of vinegars in Nutrient broth were prepared, and then 100 µL inoculum of each pathogenic bacteria (6.0 $\log_{10}$) was separately added to the tubes. Then, the inoculated tubes were kept at 35 ± 1 °C for 24 h, and the lowest concentration of the vinegars with no visible bacterial growth was accepted as MIC values (21). The MBC and MFC value was subsequently determined following the completion of the MIC assay. A 10-µL aliquot from each of the tubes containing concentrations of 2 × MIC, 4 × MIC, and 8 × MIC was spread onto MHA plates. After 24 h of incubation at 37°C, the minimum concentration that completely inactivated the microorganisms was determined as the MBC and MFC values (22).

Statistical Analyses: In the present study, the data were obtained from three independent replicates. Three different samples from each vinegar were employed in each replicate. Following preliminary statistical evaluation, it was ascertained that the data obtained satisfied the parametric test assumptions. Consequently, analysis of variance (ANOVA) and post-hoc Tukey's test was employed to ascertain the differences between the vinegars. The statistical analyses were conducted using the SPSS software, version 21.0. All data sets were presented as mean ± standard deviation, and a *p* -value of 0.05 was considered statistically significant.

Results

In this study, a total of five organic acids, including volatiles (e.g. acetic acid and butyric acid) and non-volatiles (e.g. lactic acid, malic acid and citric acid) were determined in the vinegars. However, the amount of these acids were considerably different among the vinegars, as shown in Table 1 ($p<0.05$). In this regard, the levels of citric and acetic acids in the black mulberry, malic acid in the mulberry, lactic acid in the date, and butyric acid in the pomegranate vinegars were found higher in comparison to the other vinegars ($p<0.05$). In addition, the most abundant organic acid was acetic acid, which is found in the black mulberry vinegar.

Table 1. Organic acids present in the vinegars (Mean ± S.D.)

Organic acids (g/L)	Date	Fig	Mulberry	Black Mulberry	Pomegranate
Citric Acid	0.65±0.01^{B}	0.64±0.07^{B}	0.74±0.01^{B}	10.64±0.06^{A}	0.09±0.0^{C}
Malic Acid	5.16±0.01^{B}	1.00±0.07^{E}	12.28±0.06^{A}	2.70±0.02^{C}	2.45±0.05^{D}
Lactic Acid	10.65±0.52^{A}	9.53±0.20^{B}	9.82±0.17^{B}	2.30±0.06^{D}	4.58±0.17^{C}
Acetic Acid	29.24±7.13^{B}	29.63±0.27^{B}	28.89±0.15^{B}	106.79±4.82^{A}	7.00±0.27^{C}
Butyric Acid	0.01±0.01^{D}	0.89±0.08^{B}	0.13±0.01^{CD}	0.24±0.03^{C}	3.54±0.07^{A}

$^{A-E}$: The mean values with different letters in the same line are significantly different ($p<0.05$).

Table 2. Individual phenolic and flavonoid compunds present in the vinegars (Mean ± S.D.)

	Vinegars				
Compounds (mg/L)	Date	Fig	Mulberry	Black Mulberry	Pomegranate
Gallic acid	N.D.	0.45±0.02^{CD}	1.01 ±0.06^{B}	5.34±0.40^{A}	0.91±0.01^{BC}
Protocaucic acid	1.20±0.03^{B}	1.27±0.00^{B}	1.84±0.06^{A}	0.96±0.10^{C}	0.50±0.0^{D}
Procyanidin B2	0.14±0.0^{C}	4.38±0.16^{A}	3.28±0.0^{B}	N.D.	4.25±0.02^{A}
Catechin	1.53±0.12^{D}	1.76±0.03^{C}	2.17±0.08^{B}	9.43±0.06^{A}	0.94±0.01^{E}
1.4-Dihydroxy benzoic acid	0.63±0.73^{C}	0.66±0.03^{C}	3.50±0.86^{A}	1.20±0.0B^{C}	2.03±0.05^{B}
Syringic acid	0.43±0.09^{A}	N.D.	0.13±0.03^{B}	0.41±0.17^{A}	0.13±0.0^{B}
Epicatechin	0.09±0.03^{B}	0.29±0.0^{A}	0.12±0.0^{B}	0.16±0.07^{B}	0.30±0.0^{A}
Hesperidin	N.D.	N.D.	N.D.	4.29±0.0	N.D.
Caftaric acid	3.83±0.22^{C}	4.11±0.06^{C}	12.88±0.20^{B}	34.00±0.16^{A}	3.17±0.0^{D}
Chlorogenic acid	3.50±0.02^{C}	3.48±0.01^{CD}	3.64±0.02^{A}	3.59±0.01^{B}	3.47±0.0^{D}
2-5 Dihydroxy benzoic acid	3.36±0.01^{C}	3.35±0.0^{C}	3.39±0.01^{B}	3.44±0.02^{A}	3.35±0.0^{C}
t-Caffeic acid	1.02±0.05^{B}	1.56±0.05^{A}	0.49±0.28^{C}	1.25±0.20^{AB}	0.05±0.0^{D}
p-Coumaric acid	3.73±0.01^{C}	3.73±0.0^{C}	3.77±0.0^{B}	3.80±0.01^{A}	3.73±0.0^{C}
Sinapic acid	2.83±0.0	N.D.	2.83±0.0	N.D.	N.D.
Ferulic acid	3.08±0.0	3.08±0.0	N.D.	3.10±0.02	N.D.
Resveratrol	0.41±0.0	0.41±0.0	0.41±0.0	0.41±0.0	N.D.
Rutin	1.92±0.01^{C}	2.97±0.02^{A}	N.D.	1.94±0.17^{C}	2.26±0.03^{B}
Quercetin-3-glucoside	2.82±0.04^{B}	2.78±0.0^{B}	3.67±0.02^{A}	2.86±0.10^{B}	N.D.
Kaempferol-3-glucoside	2.23±0.01^{A}	1.72±0.0^{C}	N.D.	1.90±0.0^{B}	N.D.
Myricetin	2.71±0.02^{A}	N.D.	N.D.	2.53±0.0^{B}	N.D.
Quercetin	1.95±0.03^{C}	2.63±0.01^{A}	2.47±0.0^{B}	1.96±0.0^{C}	N.D.
Luteolin	N.D.	N.D.	N.D.	5.41±1.21	N.D.
Kaempferol	1.88±0.03	N.D.	1.89±0.0	1.87±0.01	N.D.

$^{A-E}$: The mean values with different letters in the same line are significantly different ($p<0.05$). N.D.: Not Detected.

As illustrated in Figure 1, a total of 7 carboxylic acids, 21 alcohols, 19 esters, 8 ketones, 6 phenyl and phenol compounds, 5 terpenes, and 8 miscellaneous compounds were identified in the volatile fractions of the vinegars. Among the carboxylic acids, acetic acid was the most abundant compound in all vinegars. The black mulberry vinegar had the highest carboxylic acid concentration (955.77 peak area) among the vinegars used in the study. On the other hand, alcohols comprised the highest peak area in the volatile fractions of the date and fig. The ester compounds were identified in black mulberry vinegar, with a concentration of 1046.93 peak area. Additionally, these compounds were identified as the most prevalent volatiles in the black mulberry vinegar. The study revealed that ketones, phenyl and phenol compounds, as well as acids and esters, exhibited higher peak areas in the black mulberry vinegar in comparison to the other samples.

It was found that the pH values of date, fig, mulberry, black mulberry, and pomegranate vinegars were found to be 3.58, 3.74, 3.68, 3.27, and 3.59 respectively (Figure 2). TPC values of the vinegar samples were found to range between 96.74 and 982.61 mg GAE/L. The differences between the vinegars were found to be significant in terms of TPC, as shown in Figure 3 ($p \leq 0.05$). In the current study, a total of 20 different phenolic and flavonoid compounds were identified in the date, 17 in the fig and mulberry, 21 in the black mulberry, and 13 in the pomegranate vinegars, as shown in Table 2. Caftaric acid was identified as the most prevalent phenolic compound in the date, mulberry, and black mulberry vinegars, with concentrations of 3.83, 12.88, and 34.00 mg/L, respectively. Additionally, procyanidin B2 was identified as the most prominent polyphenol in fig and pomegranate vinegars, with concentrations of 4.38 and 4.25 mg/L, respectively. The characterization analysis revealed that the black mulberry vinegar had the highest concentrations of organic acids, phenolic and flavonoid compounds, phenyl and phenols, ketones, esters, and acids in comparison to the other vinegars.

The MIC, MBC, and MFC values, and the diameter of inhibition zones are shown in Table 3. It was found that the MIC values of the vinegars were found to range between 6.25 to 20.83% against the tested strains. Furthermore, the MBC and MFC values displayed a range of 6.25% to 50%. The black mulberry had the lowest MIC, MBC, and MFC values, and displayed the highest inhibition zones against the tested pathogens compared to the other vinegars ($p<0.05$). The *in vitro* antimicrobial activity tests showed that the pomegranate vinegar had the highest MIC, MBC, MFC, and the lowest diamater of inhibition zones compared to other vinegar samples.

Table 3. Minimum inhibitory concentration (%), minimum bactericidal concentration (%), and the diameter of inhibition zones (mm) of the vinegar against different pathogenic bacteria (Mean ± S.D.)

	Pathogens	Date	Fig	Mulberry	Black Mulberry	Pomegranate
MIC (%)	*Escherichia coli* O157:H7 ATCC 43895	6.25±0.0^{B}	6.25±0.0^{B}	6.25±0.0^{B}	1.56±0.0^{B}	16.67±7.22^{A}
	Escherichia coli ATCC 35218	5.21±1.80^{B}	6.25±0.0^{B}	8.33±3.61^{B}	1.56±0.0^{B}	20.83±7.22^{A}
	Listeria monocytogenes ATCC 13932	6.25±0.0^{B}	6.25±0.0^{B}	8.33±3.61^{AB}	3.13±0.0^{B}	16.67±7.22^{A}
	Salmonella Typhimurium ATCC 14028	6.25±0.0^{B}	6.25±0.0^{B}	6.25±0.0^{B}	3.13±0.0^{B}	16.67±7.22^{A}
	Salmonella Enteritidis ATCC 13076	6.25±0.0^{B}	6.25±0.0^{B}	8.33±3.61^{AB}	3.13±0.0^{B}	16.67±7.22^{A}
	Staphylococcus aureus ATCC 25923	6.25±0.0^{B}	5.21±1.80^{B}	6.25±0.0^{B}	1.56±0.0^{C}	12.5±0.0^{A}
	Aspergillus parasiticus NRRL 2999	6.25±0.0^{B}	6.25±0.0^{B}	5.21±1.80^{B}	1.56±0.0^{B}	16.67±7.22^{A}
MBC (%)	*Escherichia coli* O157:H7 ATCC 43895	18.75±8.84^{B}	18.75±8.84^{B}	50.0±0.0^{A}	9.38±4.42^{B}	50.0±0.0^{A}
	Escherichia coli ATCC 35218	18.75±8.84^{B}	25.0±0.0^{B}	25.0±0.0^{B}	6.25±0.0^{C}	50.0±0.0^{A}
	Listeria monocytogenes ATCC 13932	25.0±0.0^{B}	50.0±0.0^{A}	25.0±0.0^{B}	18.75±8.84^{B}	50.0±0.0^{A}
	Salmonella Typhimurium ATCC 14028	25.0±0.0^{B}	18.75±8.84^{BC}	25.0±0.0^{B}	9.38±4.42^{C}	50.0±0.0^{A}
	Salmonella Enteritidis ATCC 13076	18.75±8.84^{BC}	25.0±0.0^{B}	25.0±0.0^{B}	12.5±0.0^{C}	50.0±0.0^{A}
	Staphylococcus aureus ATCC 25923	25.0±0.0^{B}	18.75±8.84^{BC}	25.0±0.0^{B}	12.5±0.0^{C}	50.0±0.0^{A}
	Aspergillus parasiticus NRRL 2999	25.0±0.0^{B}	18.75±8.84^{BC}	18.75±8.84^{BC}	6.25±0.0^{C}	50.0±0.0^{A}
Inhibition Zones (mm)	*Escherichia coli* O157:H7 ATCC 43895	9.59±0.61^{BC}	9.89±0.40^{B}	9.59±0.35^{BC}	18.41±1.33^{A}	9.30±0.22^{C}
	Escherichia coli ATCC 35218	16.96±1.82^{B}	11.48±0.54^{C}	10.28±0.61^{D}	18.15±0.80^{A}	8.86±0.35^{E}
	Listeria monocytogenes ATCC 13932	9.23±0.54^{B}	9.19±0.76^{B}	8.96±0.59^{B}	17.90±1.30^{A}	9.12±0.78^{B}
	Salmonella Typhimurium ATCC 14028	10.65±0.51^{C}	12.02±0.76^{B}	11.02±0.77^{C}	18.90±0.95^{A}	9.39±0.21^{D}
	Salmonella Enteritidis ATCC 13076	9.60±0.45^{C}	10.06±0.69^{B}	10.22±0.55^{B}	18.60±0.77^{A}	9.00±0.28^{D}
	Staphylococcus aureus ATCC 25923	11.34±0.17^{C}	10.51±0.51^{D}	12.22±0.64^{B}	19.81±0.57^{A}	9.14±0.34^{E}
	Aspergillus parasiticus NRRL 2999	8.69±0.44^{C}	9.92±0.60^{B}	9.67±0.57^{B}	15.14±0.70^{A}	8.22±0.19^{D}

$^{A-E}$: The mean values with different letters in the same line are significantly different ($p<0.05$).

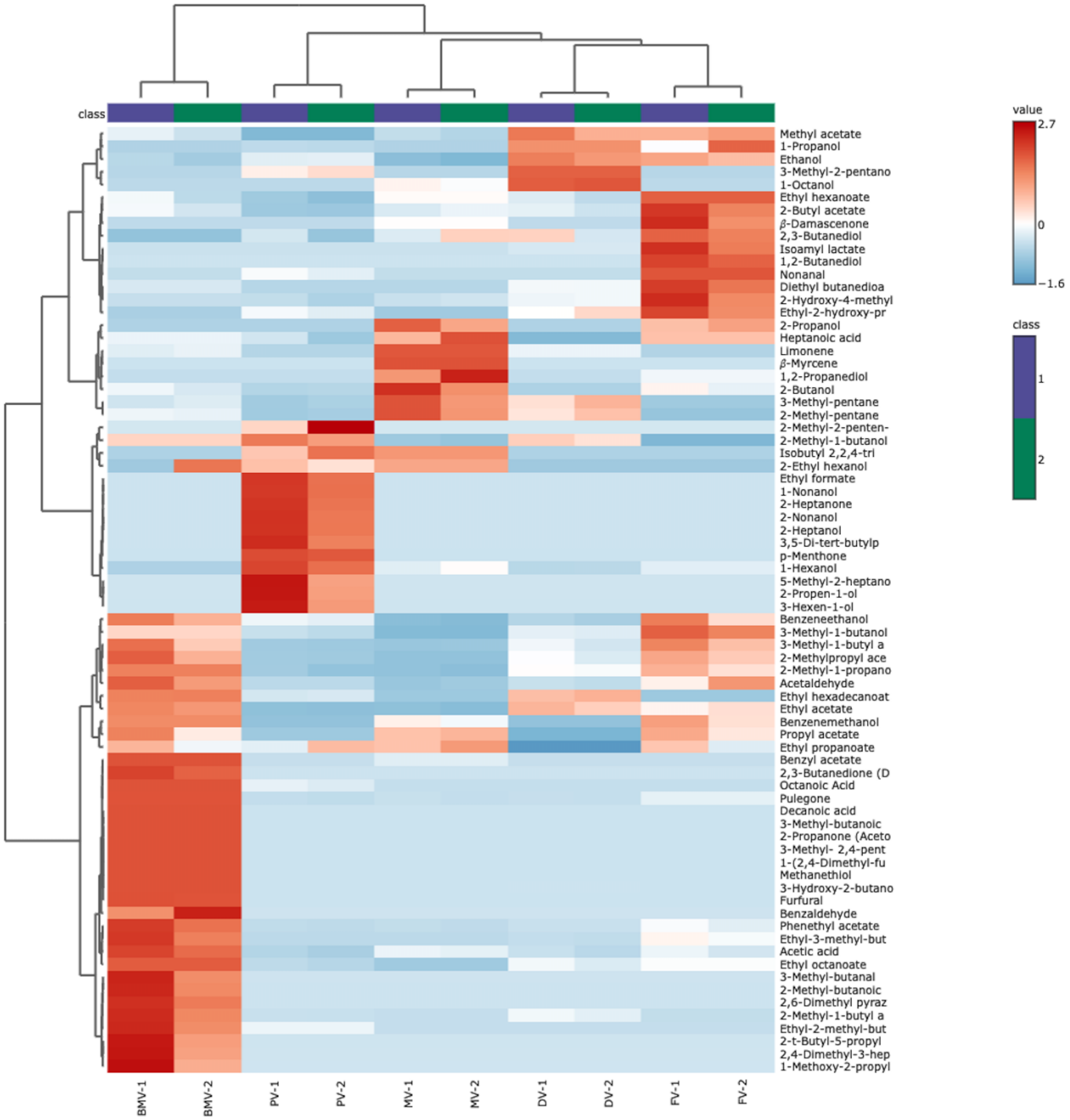

Figure 1. The hierarchical clustering analysis with a heatmap presentation of the volatiles identified in the vinegar samples used in the study

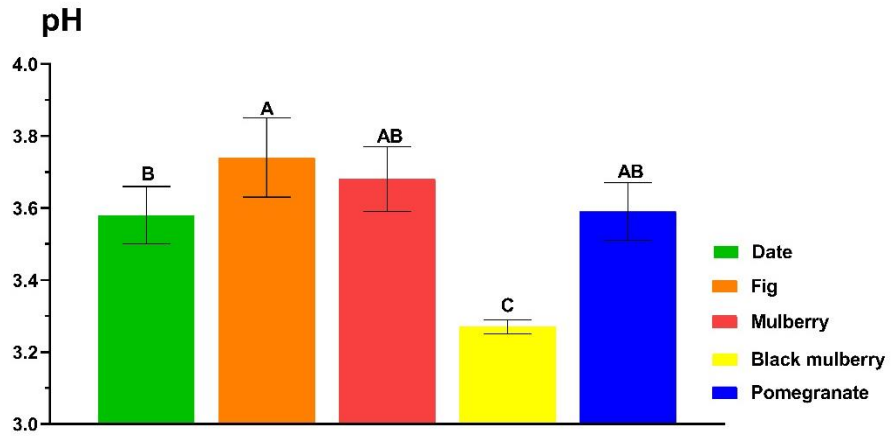

Figure 2. The pH values of the vinegars (Mean ± S.D.). [A-C]: The mean values with different letters are significantly different (p<0.05)

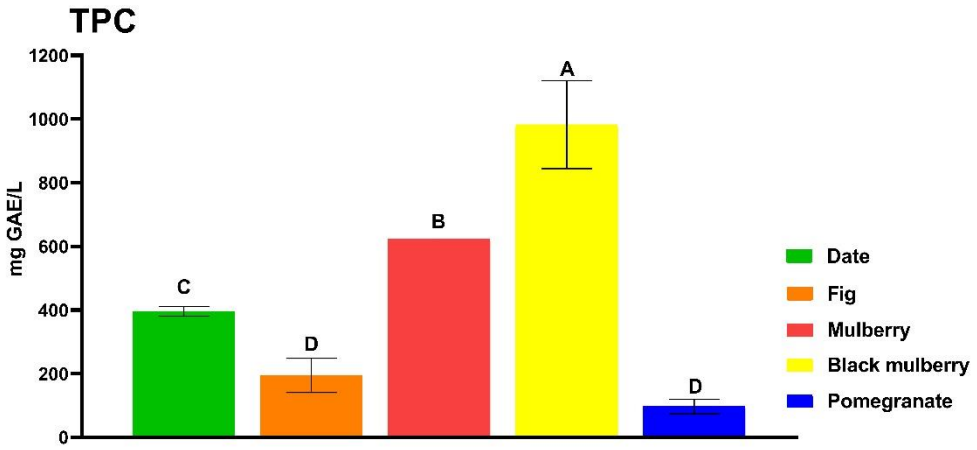

Figure 3. The total phenolic content (TPC) values of the vinegars (Mean ± S.D.). [A-D]: The mean values with different letters are significantly different (p<0.05).

Discussion

The organic acids present in vinegars are not only considered exclusively as nutritional constituents; they also exhibit a wide range of bioactive properties. It has been demonstrated that certain organic acids, including malic, citric, and lactic acids, are capable of entering the tricarboxylic acid cycle, which represents the primary metabolic pathway for the three main nutrients (carbohydrates, lipids, and amino acids). Moreover, the organic acids are primarily responsible for the acidic pH values of the vinegars, and they also contribute to the antimicrobial properties. It was demonstrated that acetic acid, among other compounds, is capable of permeating microbial cell membranes, resulting in bacterial cell death (3, 8, 23). In aggerement with this information, the result of this study showed that the black mulberry, which exhibited the highest acetic acid concentration, displayed the most pronounced antimicrobial properties compared to the other vinegar samples.

In this study, a total of five organic acids were identified in the vinegars, with significant differences in their concentration between the samples. Similarly, other studies have documented comparable variations in the organic acid composition of the vinegars (3, 5, 24).

It is well known that volatile compounds exert a significant influence on the sensory attributes of foods and consumer acceptance (25). As mentioned above, the level of these volatiles is primarily depends on the raw material used in the vinegar production and the fermantation conditions. In line with this information, the results of this study demonstrated that the vinegars comprised a considerable quantity of volatiles. In addition, alcohols and esters, which are produced through the fermentation of sugars by microorganisms, are known for display a diverse array of bioactivities. These include antimicrobial, anticancer, and antitumour properties (8). A variety of volatile compounds have been identified in a number of vinegars produced by different fruits, including black mulberry, white mulberry, hawthorn, grapes, apple

etc. The studies have revealed that the vinegar produced from a given fruit can exhibit a distinctive profile of volatile compounds, which may be influenced by factors such as the variety of fruit, the processing method, and the fermentation conditions (8, 16, 19, 25-27). These pieces of information can explain the differences in the levels of the bioactive compounds and bioactivies of the vinegars used in the current study.

The pH values of date, fig, mulberry, black mulberry, and pomegranate vinegars were found between 3.27 and 3.74. Consistently, in a study conducted by Silva et al. (28), the pH values of the six different vinegar samples were found to range between 2.83 and 3.49. Furthermore, Kara et al. (6) reported that the pH values of the 11 vinegars were found to be between 2.37 and 4.47. As shown in Figure 2, the black mulberry vinegar had the lowest pH (3.27±0.02). Contrarily, Kara et al. (5), reported that the four apple vinegars had the lowest pH (3.6). In addition, in a study conducted by Yıldız et al. (9), the pH value of whey vinegar was found to be 4.55. As demonstrated by the literature reviewed above, there are considerable variations in the pH values of the various vinegars. The possible reasons for this phenomenon are thought to be attributable to the type of fruit used in vinegar production or the method practised in vinegar production process (19).

As shown in Figure 3, significant differences were found in the levels of TPC among the vinegars, and the highest TPC was found in the black mulberrry ($P \leq 0.05$). The results revealed that the vinegars varied significantly in terms of TPC, and this finding is consistent with the existing literature (6, 28). It is well known that phenolics and flavonoids have antimicrobial and antioxidant properties. Furthermore, flavonoids have been demonstrated to exhibit a variety of bioactive effects, including anti-inflammatory, anti-thrombotic, and anti-mutagenic activities. (8). These compounds have also affect on the sensory characteristics of vinegars (16). The phenolic compounds present in vinegar are mainly derived from the raw materials used in its production. Furthermore, it has been demonstrated that the total polyphenol concentration in vinegar increases in line with the duration of the ripening process (3, 10, 29, 30). In the current study, a total of 20 different phenolic and flavonoid compounds were identified in the date, 17 in the fig and mulberry, 21 in the black mulberry, and 13 in the pomegranate vinegars (Table 2). The reasons for the differences in the variety and quantity of polyphenols identified in the various vinegar samples may be associated with the aforementioned factors.

The MIC, MBC, and MFC values, as well as the diameter of inhibition zones of the vinegars, were found to range between 1.56 and 20.83%, 6.25 and 50%, and 8.22 and 19.81 mm, respectively, against the tested microorganisms. In contrast, Sengün et al. (31) reported that the MIC values of the different vinegars were found to range betwee 3.12 to 12.5% against certain foodborne pathogens. In another study, Kahraman et al. (19) found that the MIC values of apple and grape vinegars against eleven pathogens were ranged from 6.25% to12.5%, and the MBC values were ranged from 6.25 to 25%. In addition, it was found that the MIC values of the vinegars were recorded as 1.95 and 500 µL/mL against *S. aureus* and *C. Albicans*, respectively (6). However, they also reported that the *Aspergillus parasiticus* exhibited resistance against all of the vinegars (6). Yıldız et al. (9) reported that the MIC and MBC values were found to be 6.25 and 50 µL/mL against seven pathogenic microorganisms. On the other hand, Kara et al. (5) tested the antimicrobial effect of the four different apple vinegars against five pathogenic microorganisms, and the MIC values were found between 1.95 and 500 µL/mL, and the MBC values were ranged from 3.91 and 500 µL/mL.

In our previous study, the diameters of inhibition zones of the hawthorn vinegar were ranged from 9.22 to 11.43 mm against 12 pathogenic bacteria (8). In different studies, the diamater of inhibition zones of the various vinegars were foudn to be range between 6.30 to 40.17 mm (5, 6, 9, 28). It is believed that the differences between the studies are probably due to the pH value of the vinegars, the level of bioactive compounds that contribute to the antimicrobial activity (organic acids, volatiles, and polyphenols, etc.), and the susceptibility of the tested microorganisms.

The characterization analyses of the vinegars revealed that all vinegars used in the study contain a wide range of bioactive compounds, including organic acids, individual phenolic and flavonoid compounds, and volatiles. Nevertheless, the quantities of these bioactive compounds exhibited considerable variation between the vinegars. Consequently, the vinegars exhibited a diverse spectrum of antimicrobial activity against the tested microorganisms. Among the vinegars, black mulberry exhibited highest antimicrobial activity in comparion to the other vinegars. The result of the current research indicate that the vinegars displayed a considerable potential in terms of *in vitro* antimicrobial activity. Although they have a strong antimicrobial effect, their use in foods should take into account that they may have a pungent taste and aroma specific to the fruit from which they are derived. In conclusion, vinegars can be employed as a natural preservative to enhance the microbiological quality and safety of foods.

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