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TOTAL PHENOLIC CONTENT AND ANTIBACTERIAL ACTIVITY OF HOMEMADE FIG AND MULBERRY VINEGAR

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ABSTRACT

In this study, antimicrobial activity of traditionally produced fig and mulberry vinegar was examined. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of vinegar samples against eight microorganisms including *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pediococcus acidilactici* were determined. Both types of vinegar exhibited inhibitive effect on all test microorganisms. The MIC values of fig and mulberry vinegar were determined as ranging between 0.39-12.5% and 3.12-12.5% (v/v), respectively. The most sensitive bacteria to fig vinegar was *B. subtilis* while *S. typhimurium* was the most sensitive one to mulberry vinegar. After neutralization of vinegar, bactericidal effect was observed only in fig vinegar sample, which had higher total phenolic content than mulberry vinegar. These results indicated the potential of home-made vinegars as antimicrobial substance that could be used as functional food ingredients and as food supplements.

Keywords: Fig, Mulberry, Homemade vinegar, Antimicrobial, Total phenolic contents

EV YAPIMI İNCİR VE DUT SİRKESİNİN TOPLAM FENOLİK İÇERİĞİ VE ANTİBAKTERİYEL AKTİVİTESİ

ÖZET

Bu çalışmada, geleneksel olarak üretilen incir ve dut sirkesinin antimikrobiyal aktivitesi incelenmiştir. Sirke örneklerinin minimum inhibisyon konsantrasyonu (MİK) ve minimum bakterisidal konsantrasyonu (MBK) *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* ve *Pediococcus acidilactici*'yi içeren sekiz mikroorganizmaya karşı belirlenmiştir. Her iki sirke çeşidi de tüm test mikroorganizmaları üzerinde inhibitif etki göstermiştir. İncir ve dut sirkesinin MİK değerlerinin sırasıyla %0.39-12.5 ve %3.12-12.5 (h/h) arasında değiştiği belirlenmiştir. İncir sirkesine karşı en hassas bakteri *B. subtilis* iken, *S. typhimurium* dut sirkesine karşı en hassas bakteri olmuştur. Nötralize edilen sirkelerde bakterisidal etki, toplam fenolik içeriği dut sirkesinden daha yüksek olan incir sirkesi örneğinde gözlenmiştir. Bu sonuçlar, antimikrobiyal madde olarak ev yapımı sirkelerin fonksiyonel gıda bileşenleri ve gıda takviyesi olarak kullanılabilme potansiyelini göstermiştir.

Anahtar kelimeler: İncir, Dut, Ev yapımı sirke, Antimikrobiyal, Toplam fenolik içerik

1. INTRODUCTION

Vinegar is a special kind of condiment produced from a variety of raw materials containing fermentable carbohydrates through the activity of yeasts and acetic acid bacteria. During vinegar fermentation, organic acids, predominantly acetic acid, which is responsible for the basic sensorial characteristic of vinegar, are produced by acetic acid bacteria [1]. It has long been used not only as flavoring and preserving agent, but also used in traditional and natural folk medicine for treating a variety of diseases.

Several researchers demonstrated that the vinegars effectively inhibit the growth of foodborne pathogens including *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella typhi*, *S.* Typhimurium, *Staphylococcus aureus*, *Vibrio parahaemolyticus* and respiratory pathogens such as *Micrococcus catarrhalis*, *Staphylococcus albus*, *Diplococcus pneumonia* and *Alpha streptococcus* [2, 3]. Vinegar could be used for disinfection of a variety of equipment, foods and food preparation surfaces.

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Furthermore, pathogenic bacteria were also successfully eliminated from vegetables by vinegar rinsing or soaking [4-9].

Vinegars can mainly be grouped as grain and fruit vinegars, based on raw materials used during production [10]. Fig (*Ficus carica* L. (Moraceae)) and mulberry (*Morus alba* (Mora)) are important fruits native in Anatolia and grown in large areas in Turkey [11, 12]. The fruit and shell parts of fig, which has high amount of phenolic content, could also be used as natural antioxidant in food and pharmaceutical industries [13, 14]. Fresh and/or dried fig could be used as raw material for traditional vinegar production in Turkey. The quality properties of fig vinegar show differences depending on the raw material and production techniques used [15]. Mulberry, which has high nutritional value and attractive taste, could also be used for traditional vinegar production like fig vinegar. It was reported that mulberry vinegar contains higher amount of lactic and succinic acids than other fruit vinegars and have potential antioxidant and antimicrobial effects [16, 17, 18].

The interest for traditionally produced vinegars is now growing among the consumers. Vinegars are known to have several physiological functions, especially those made by traditional techniques [19]. Total phenolic contents of vinegars, which show differences depending on raw material and production method used, indicate antioxidant antimicrobial potential of the product [1]. There are several studies on traditional grape and apple vinegar, which interpreting the phenolic contents and antioxidant activities of the vinegars. However, to the best of authors' knowledge, no study is found on the relationship between the total phenolic contents and antimicrobial activities of fig and mulberry vinegar. Therefore, the objectives of this study were; 1) to investigate the antimicrobial properties of traditionally produced fig and mulberry vinegars against foodborne microorganisms by determining minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs), 2) to determine the relationship between antimicrobial activity and total phenolic contents of vinegars.

2. MATERIALS AND METHODS

2.1. Vinegar Samples

Vinegar samples, produced traditionally at homes were used as analyze samples. Fig vinegar was produced from fresh fig in Aydın, Turkey. The same receipt was used for mulberry vinegar production in Kars, Turkey, only replacing the fig with mulberry (Figure 1). Collected samples were stored at 4°C before used in the experiments.

Fresh fruit or vegetable+Water (1:1, w/v)
↓
First fermentation (at room temperature, 2-3 weeks)
↓
Filtration
↓
Second fermentation (at room temperature, 10-12 weeks)
↓
Bottling

Figure 1. Production of traditional vinegar [15].

2.2. Determination of pH and Acidity

The pH value of each sample was measured using a pH meter (NEL Mod 821). The total acidity of the vinegars was determined by titrimetric method and the results were expressed as g acetic acid/100 ml sample [20]. Experiments were conducted in three replicates.

2.3. Determination of Total Phenolic Contents

Total phenolic contents in vinegars were determined by the Folin-Ciocalteau colorimetric method [21]. 75 ml of distilled water was mixed 1 ml of vinegar sample and 5 ml of Folin-Ciocelteau's phenol reagent (10%). After the mixture was kept for 3 min at room temperature, 10 ml saturated Na_2CO_3 (75 g/l) was added in the mixture. The final mixture was completed to 100 ml with distilled water and incubated at the room temperature in the dark for 90 min. Then the incubation absorbance of the mixture was measured using a spectrophotometer (Agilent Technologies, Carry60 UV-Visible) at 720 nm. Total phenolic contents were expressed as mg gallic acid equivalents (GAE)/l. Gallic acid calibration curve was obtained with different gallic acid concentration in ethanol. Experiments were conducted in three replicates.

2.4. Determination of Antimicrobial Effect

Antimicrobial activity of vinegar samples was determined by detecting minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC).

2.4.1. Preparation of bacterial strains

Listeria monocytogenes Scott A, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6037, *Staphylococcus aureus* ATTC 6538P, *Escherichia coli* O157:H7 ATCC 43895, *Salmonella typhimurium* NRRL-B-4420, *Escherichia coli* ATCC 1103 and *Pediococcus acidilactici* ATCC 8042 were used as test cultures for evaluating the antimicrobial activity of vinegar samples. In the study, it is mainly focused on the pathogens associated with food-borne diseases, but representative species for food spoilage (*B. subtilis*) and lactic acid bacteria (*E. faecalis* and *P. acidilactici*) have also been included.

All microorganisms were obtained from Food Microbiology Laboratory, Food Engineering Department, Ege University, Turkey. The bacterial cultures stored at -20°C were regenerated for several times in Mueller-Hilton Broth (MHB, pH 7.3±0.2, CM405-Oxoid, Basingstoke, Hampshire, England) at 37ºC for 18-24 hr. The optimized bacterial cultures (DEN-1 McFarland Densitometer, Grant-bio), equivalent to 0.5 McFarland turbidity standard, were used in the analyses.

2.4.2. Determination of minimum inhibition concentration (MIC) of vinegar samples

The MIC value of the vinegars was determined by microdilution method using standard 96-well microtiter plates, according to the modified protocol described by [22]. Serial two-fold dilutions of the vinegars prepared with MHB (a total volume of 200 µl), were dispensed into wells of the microplate. In wells of the prepared microplate, the final concentrations of the vinegar were; 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.20%, 0.10% (v/v), respectively. After dilution of the samples, 10 µl of the bacterial culture was inoculated into each well. Wells containing only MHB and the test cultures were used as negative and positive controls, respectively. The dilution and inoculation procedure described was repeated for each vinegar sample and for each test microorganism, separately. After incubating the plates at 37ºC for 18 hr, 20 µl of 0.5% (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC, Merck, 108380, Germany) aqueous solution was added into the wells and the color change of the wells were interpreted after 30 min at 37ºC. The lowest concentration of the vinegar required to inhibit visible growth of the test culture (no color formation) was selected as the MIC value.

2.4.3. Determination of minimum bactericidal concentration (MBC) of vinegar samples

After detecting the MIC values, samples were taken from all the wells where no bacterial growth (no color change) was observed and streaked on Mueller-Hilton Agar (MHA, pH 7.3±0.2, CM337-Oxoid) to determine MBCs of vinegars. The plates incubated at 37ºC for 24 hr were checked for colony formation [23]. The results of MBC values of vinegar samples were given as % (v/v).

2.4.4. Determination of the effect of pH on test microorganisms

Vinegar samples were neutralized to detect whether or not the antimicrobial effect depends on the acid content of the vinegars. The vinegar samples were aseptically neutralized with 1 N NaOH (PubChem CID: 14798, Merck). Then, the MIC and MBC values of neutralized vinegar samples were determined against test cultures by the same method described above.

3. RESULTS AND DISCUSSION

3.1. pH and Acidity of Vinegars

The pH value of vinegars was determined as 3.75±0.21 for fig vinegar and 2.87±0.43 for mulberry vinegar. The total acidity of fig and mulberry vinegar was found as 3.67±0.35 and 4.07±0.16 g acetic acid /100 ml sample, respectively. Hence, total acidity of mulberry vinegar was found higher than fig vinegar (Table 1). In the previous study, the pH value and total acidity of the fig vinegars ranged from 3.05 to 3.73 and 2.10 to 6.97 g acetic acid/100 ml, respectively [15]. In the study carried by [24], the total acidity of mulberry vinegar was determined as 5.72 g acetic acid/100 ml. These results indicated that pH and acidity values of vinegars can vary in a wide range depending on the type of raw material.

Table 1. The pH, total acidity and total phenolic contents of vinegar samples

Item	Fig Vinegar	Mulberry Vinegar
pH	3.75±0.21^{a}	2.87±0.43^{a}
Total Acidity * (g /100 ml)	3.67±0.35^{a}	4.07±0.16^{a}
Total Phenolic Content (mg GAE/l)	767±8.48^{a}	557.5±28.99^{b}

*: as acetic acid percentage. Standart deviation of means are shown as ± SD. Values in the same row with different superscripts are statistically different ($P<0.05$).

3.2. Total Phenolic Contents of Vinegars

The total phenolic contents in fig and mulberry vinegars were determined by a colorimetric assay, using the Folin–Ciocalteu reagent. The result showed that total phenolic content of fig vinegar (767±8.48 mg GAE/l) was higher than mulberry vinegar (557.5±28.99 mg GAE/l) (Table 1). It was previously detected that the total phenolic contents in mulberry vinegar was 972.708 mg GAE/l [24]. Moreover, no references concerning the total phenolic content of fig vinegar could be found despite the thorough literature survey. In the study performed by [25], the total phenolic contents of the traditional vinegars ranged from 75.01 to 2228.79 mg GAE/l for grape vinegars and 40.44 to 434.88 mg GAE/l for apple vinegars. These results showed that large differences exist in the total phenolic contents of vinegars, which are mainly originated from raw material. A number of studies have concluded that the production method of vinegar also affects the total phenol contents of vinegars [26-29]. In a study, phenolic content of wine vinegars produced by traditional surface and industrial submerged methods were determined. Total phenolic content of traditional and industrial vinegar samples were 2690 mg/l and 2461 mg/l GAE, respectively. The content of catechin from phenolic substances in industrial vinegar (27.50 mg/l) was significantly higher than that of in traditional vinegar (13.76 mg/l), while traditional vinegar had higher amounts of chlorogenic and syringic acids than the industrial vinegar [27]. Also, phenolic contents in vinegar samples may derive from the raw material or may be obtained to it by aging of the vinegar in wooden barrels. Therefore, large differences exist in the content of phenolic compounds among different kinds of vinegars. In another study, catechin was not found in malt vinegar, while 8.29 mg catechin/100 ml was observed in apple vinegar [26]. These results indicate that the polyphenolic content may change between different vinegars.

In addition, the types of phenolic constituents found in different types of vinegars may change. Grape and apple vinegar contain predominantly gallic acid, catechin, epicatechin, caffeic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid and *p*-hydroxybenzoic acid. On the other hand, phenolic compounds of apricot, artichoke, blackberry, lemon, pomegranate, rice and rosehip vinegar were defined by various researchers as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, catechin, vanillic and syringic acid [27, 30]. Besides, gallic acid, caffeic acid and *p*-coumaric acid were found in balsamic vinegar, while blueberry vinegar was contained only gallic acid and protocatechuic acid from phenolic compounds [30]. In the literature, there are only one study that determined the phenolic compounds found in mulberry vinegar [30]. In this study, it was determined that mulberry vinegar contain phenolic compounds such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, catechin, vanillic acid and caffeic acid. However, no studies have been conducted to detect phenolic compounds in fig vinegar. It is known that phenolic compounds found in foods have antioxidant and also antimicrobial activities [31, 32]. Specifically, gallic acid is reported to have a strong antimicrobial activity [33]. It was reported that the strong bactericidal effect of balsamic vinegar may be related to the compounds with antimicrobial properties resulting from the fermentation of grape juice and grape juice itself. It was shown that grapes and grape-based products contain a number of phenolic compounds that exhibit antibacterial activity, particularly phenolic compounds such as resveratrol, vanillic acid, caffeic acid and gallic acid [34, 35, 36].

3.3. The MIC and MBC Values of Vinegars

The MICs of vinegar samples were determined against eight microorganisms using a 96-well microtiter plate method, containing the final concentrations of vinegar in the range of 0.10%-50% (v/v). 12.5 percentage of vinegar was found inhibitive for all microorganisms tested. However, the results of the study showed that fig and mulberry vinegars have different MIC and MBC values (Table 2). The MICs were ranged between 0.39%-12.5% for fig vinegar and 3.12-12.5% for mulberry vinegar. Fig vinegar was shown higher antimicrobial activities than mulberry vinegar against test microorganisms, except *P. acidilactici*. The most sensitive bacteria to fig vinegar was found as *B. subtilis* with MIC of 0.39% and *S. typhimurium* for mulberry vinegar with MIC of 3.12%. *P. acidilactici* was the most resistant bacteria against vinegars (Table 2).

Table 2. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of vinegar samples (%, v/v)

	MIC Values		MBC Values	
Microorganisms	**Fig Vinegar**	**Mulberry Vinegar**	**Fig Vinegar**	**Mulberry Vinegar**
Bacillus subtilis	0.39	6.25	3.12	>50
Enterococcus faecalis	1.56	6.25	12.5	25
Escherichia coli	3.12	6.25	50	>50
Escherichia coli O157:H7	1.56	6.25	>50	>50
Listeria monocytogenes	1.56	6.25	12.5	12.5
Salmonella typhimurium	1.56	3.12	25	>50
Staphylococcus aureus	6.25	12.5	50	>50
Pediococcus acidilactici	12.5	12.5	>50	>50

Mulberry vinegar was not shown bactericidal effect against microorganisms, except *L. monocytogenes* and *E. faecalis*. The highest bactericidal effect was observed in fig vinegar against *B. subtilis* with MBC of 3.12% (Table 2). In a study performed by [18], antimicrobial activity of mulberry vinegar produced traditionally in Erzurum city, in Turkey were tested on *S. aureus, Streptococcus pyogenes, Klebsiella oxytoca, E. faecalis, B. subtilis, B. cereus, Erwinia carotovora, E. coli* and *Candida albicans* by disk diffusion method and the largest inhibition zones were observed in *S. aureus* (28 mm), *K. oxytoca* (24.6 mm) and *B. subtilis* (23.3 mm). In another study, various microorganisms (*B. cereus*, *E. coli*, *E. coli* O157:H7, *Klebsiella pneumoniae*, *L. monocytogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris, S.* Typhimurium, *S. aureus, Yersinia enterocolitica*) had sensitivity to traditionally produced vinegars (grape, apple, lemon, artichoke, pomegranate, hawthorne) at varying rates with inhibition zones ranging between 6.18 and 23.56 mm and *B. cereus* was observed as the most sensitive strain [25]. In some studies, antimicrobial activities of apple, gilaburu, blackberry, artichoke, lemon, rosehip, hawthorn, blueberry, apricot, rice and pomegranate vinegar produced by traditional methods were determined against *E. coli*, *S*. Typhimurium, *S. aureus* and *Candida albicans* [30, 37]. Although there are these studies investigating the antimicrobial effects of traditionally produced vinegars, no data was found in the literature on MICs of mulberry and fig vinegar.

3.4. The Effect of pH on Antimicrobial Activity of Vinegars

Although the acidity of fig vinegar was lower than mulberry vinegar, the antimicrobial activity of fig vinegar was found higher than mulberry vinegar, in terms of MICs and MBCs. Thus, in the study, vinegar samples were neutralized to detect whether or not the antimicrobial effect depends on the acid content of the samples. As it was expected, antimicrobial effects of vinegar samples were significantly decreased after neutralization. However, the decrease was not in the same level for both vinegar type (Table 3). The MICs of neutralized fig vinegar ranged between 1.56% and 50% while mulberry vinegar was found inhibitive at 50% concentration, except *L. monocytogenes* and *E. faecalis*. The highest inhibitive effect was observed in neutralized-fig vinegar against *E. coli* O157:H7 and *S. typhimurium* with MIC of 3.12% (Table 3).

Table 3. The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of neutralized-vinegar samples (%, v/v)

	MIC Values		MBC Values	
Microorganisms	**Neutralized-Fig Vinegar**	**Neutralized-Mulberry Vinegar**	**Neutralized-Fig Vinegar**	**Neutralized-Mulberry Vinegar**
Bacillus subtilis	50	50	50	>50
Enterococcus faecalis	12.5	25	50	>50
Escherichia coli	25	50	50	>50
Escherichia coli O157:H7	1.56	50	>50	>50
Listeria monocytogenes	25	25	>50	>50
Salmonella typhimurium	1.56	50	50	>50
Staphylococcus aureus	25	50	50	>50
Pediococcus acidilactici	12.5	50	>50	>50

-: not detected

The MBC values of neutralized vinegar were significantly increased according to original vinegar samples. Neutralized fig vinegar was bactericidal at 50% concentration on test microorganisms, except *L. monocytogenes*, *E. coli* O157:H7 and *P. acidilactici.* However, no bactericidal effect was observed in neutralized mulberry vinegar (Table 3). This result could be linked with the amount of total phenolic content of vinegars, which was found higher in fig vinegar than in mulberry vinegar. In the previous study, the antimicrobial activity of balsamic vinegar has been connected to the presence of phenolic compounds that possess antimicrobial properties [9]. Depending on the strains of acetic acid bacteria found in vinegar production, some bioactive compounds may also be produced during vinegar production [1]. Hence, types of vinegars affect their antimicrobial properties.

4. CONCLUSION

This is the first study reporting the MIC/MBC values of fig and mulberry vinegar by correlating the antimicrobial effects with the total phenolic contents of the vinegars. In the study it was determined that the inhibitive effect of fig vinegar was the highest on *B. subtilis* and the lowest on *P. acidilactici.* On the other hand, *S. typhimurium* was found the most sensitive bacteria to mulberry vinegar while *S. aureus* and *P. acidilactici* were the most resistant species. Except *P. acidilactici*, the antimicrobial activity of fig vinegar was found higher than mulberry vinegar, in terms of MICs and MBCs. This study showed that vinegars made from various raw materials have different antimicrobial activities. Although the antimicrobial activity of vinegar mainly comes from the acid content of the vinegar, the total phenolic contents of samples were also contributed this activity. Further studies are in progress to identify the bioactive components of these vinegars.

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