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THE ANTIMICROBIAL ACTIVITY OF APPLE CIDER VINEGAR AND GRAPE VINEGAR, WHICH ARE USED AS A TRADITIONAL SURFACE DISINFECTANT FOR FRUITS AND VEGETABLES

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ABSTRACT. Apple cider vinegar and grape vinegar are traditional surface disinfectants, which are commonly used in disinfection of fruit and vegetables at homes in Turkey. In this study, the antimicrobial activity of apple cider vinegar and grape vinegar are tested against standard strains (*Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044) and food isolates (*Enterococcus durans*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Listeria innocua*, *Salmonella infantis* and *Salmonella kentucky*) by minimum inhibitory concentration (MIC) test and the results are compared against Halamid®, a commercial surface disinfectant, which can be used for fruit and vegetable disinfection. As a result, it was observed that grape vinegar presented the highest activity with a MIC value of 12.5 - 50 µg/mL against all microorganisms, where the least activity with a MIC value of 25 - 50 µg/mL against all microorganisms except *C. albicans*, *E. coli*, *E. durans*, *E. faecalis*, *S. kentucky* and *P. fluorescens*.

1. INTRODUCTION

The term quality can be defined as the degree of high standard and excellence [1]. Quality of fruits and vegetables can be described by several parameters, such as the colour and the appearance, the flavour (aroma and taste), the texture and the nutritional value [1]. Several external and internal factors, such as microorganisms, enzymes, air, light, temperature and etc., may cause fruits and vegetables to deteriorate and develop some unpleasant texture, taste and odour, which is known as food spoilage [2]. Consuming spoiled food leads to food poisoning [3]. Food poisoning is one of the major problems, which is related to both economic development and public health. Food borne diseases affect countries in both developed and developing all over the world [4]. As it was mentioned previously, microorganisms are one of the factors, which are involved in food spoilage. Many pathogenic and saprophytic microorganisms are known to cause food spoilage and thus responsible of food-borne diseases [5]. Clean and reliable food can only be achieved with an effective sanitation procedure [6].

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There are several methods, which are commonly used in food sanitation and using vinegar is one of the methods that is usually preferred for food sanitation at home. Vinegar is not only used for food sanitation, but also used both in preparation of mayonnaise and salad dressings to add its characteristic flavour to the food and preserve the food for a long time [7,8].

Several studies previously documented the composition and health benefits of vinegar [9]. It is known that apple cider vinegar contains gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, and p-coumaric acid [10] and the grape vinegar contains gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, syringic acid, and ferulic acid [11]. Due to these bioactive substances vinegar has several functional therapeutic properties such as antibacterial activity, reducing high blood pressure, antioxidant activity and prevention of cardiovascular disease [12-18].

Halamid® is one of the commercial chemicals that can be used for sanitation of fruits and vegetables.

In this study, the antimicrobial activity of apple cider vinegar and grape vinegar are tested against several gram positive and gram negative bacteria and one yeast, and the results are compared against Halamid®.

2. Materials And Methods

2.1. Vinegar Samples

The apple cider vinegar and grape vinegar samples used in this study are purchased from the local supermarket, which are natural and additive free. 500 mL of each vinegar sample is attached to a freeze dryer (Christ, Germany) and the water content present in the vinegar samples were totally evaporated at 0.12 atm and - 82 ℃. At the end of the freeze drying process 9 g of sample was obtained from apple cider vinegar, where this was 14 g for grape vinegar. A stock solution of 1 mg/mL was prepared by distilled water for each sample and these stock solutions were sterilised by filtering through 0.45 µm filter.

2.2. Microorganisms

Bacillus subtilis DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans* (food isolate), *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* (food isolate), *Escherichia coli* ATCC 25922, *Klebsiella*

pneumoniae (food isolate), *Listeria innocua* (food isolate), *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis* (food isolate), *Salmonella kentucky* (food isolate), *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044 were used to test the antimicrobial activity of vinegar samples.

2.3. Preparation of Inocula

All bacterial strain were incubated at 37 ˚C for 24 hours, however *C. albicans* was incubated at 27 ˚C for 48 hours [19,20]. Each bacteria and yeast were transferred into 0.9% sterile saline solution and adjusted to 0.5 McFarland standard, in order to standardize the inocula to contain about 10^8 cfu/mL for bacteria and 10^7 cfu/mL for *C. albicans* [21,22].

2.4. Minimum Inhibitory Concentration (MIC) Test

Broth dilution method for minimum inhibitory concentration (MIC) determination as described previously was employed [23,24]. Serial 2-fold dilutions were made to obtain a concentration range of 0.195 - 100 µg/mL. The MIC was defined as the lowest concentration of extract inhibiting any visible bacterial growth. All tests were conducted in triplicates.

2.5. Controls

Mueller Hinton Broth (MHB) and microorganism inoculated MHB were used to control both the sterility of the culture medium and whether microorganisms were alive or not. In addition to those sterile distilled water, was used as negative control. On the other hand, Halamid® was used as positive control and the stock solution for Halamid® was prepared as 1 mg/mL by using distilled water and sterilised by filtering through 0.45-µm filter as in vinegar samples.

2.6. Statistics

The statistical analysis was done using a parametric method, the one-way analysis of variance (ANOVA), with a significance level of 0.05. All statistical analysis were conducted by using R Studio, version 3.3.2 [25].

3. Results And Discussion

Antimicrobial activity of apple cider vinegar, grape vinegar and Halamid® were analysed and the results of the MIC tests are given in Table 1 Figure 1.

Table 1. MIC values for apple cider vinegar, grape vinegar and Halamid® against microorganisms tested (µg/mL)

Microorganism	ACV*	GV*	Halamid®
B. subtilis	50	25	50
C. albicans	-	50	-
E. aerogenes	50	50	50
E. durans	-	25	50
E. faecalis	-	50	50
E. faecium	25	25	50
E. coli	-	25	50
K. pneumoniae	50	25	50
L. innocua	50	25	50
L. monocytogenes	50	25	50
P. aeruginosa	50	12.5	50
P. fluorescens	-	50	25
S. enteritidis	50	25	50
S. infantis	50	25	50
S. kentucky	-	25	50
S. typhimurium	50	25	50
S. aureus	25	12.5	50
S. epidermidis	25	12.5	50

* ACV: Apple cider vinegar, GV: Grape vinegar
"-" implies no activity

In all tests MHB was observed to be sterile and microorganisms were reproducing without any problems. Furthermore, the statistical analysis proved that there was no significant difference between the activities of three parallels ($p > 0.05$).

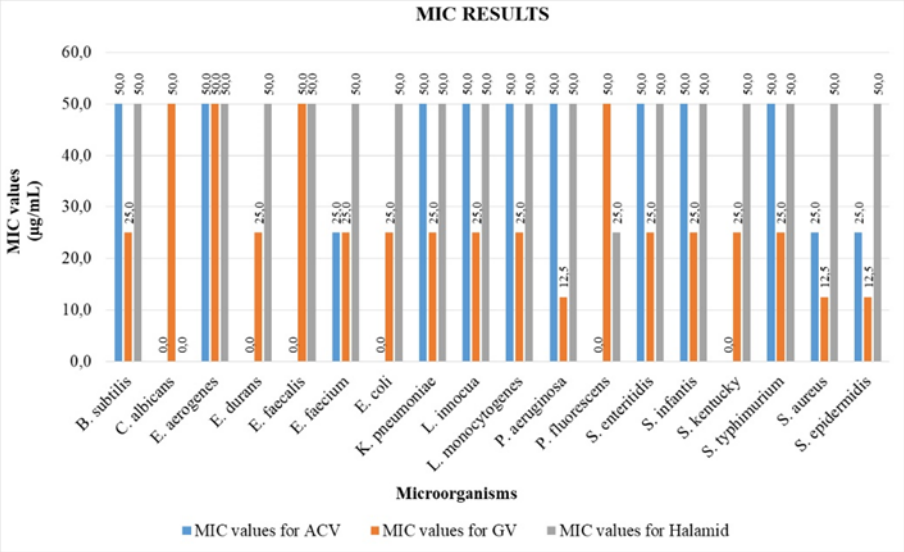

FIGURE 1. MIC values for apple cider vinegar (ACV), grape vinegar (GV) and Halamid® (µg/mL)

According to the results it was observed that apple cider vinegar affected all microorganisms except *C. albicans*, *E. coli*, *E. durans*, *E. faecalis*, *S. kentucky* and *P. fluorescens* with MIC values either 25 µg/mL or 50 µg/mL. Grape vinegar was observed to be active against all microorganisms with MIC values ranging between 12.5 and 50 µg/mL. On the other hand, Halamid® was found to be active against all microorganisms except *C. albicans* with a MIC value of 50 µg/mL, except *P. fluorescens* with a MIC value of 25 µg/mL.

There are several studies regarding the antimicrobial activity of different types of vinegar in the literature.

Chang and Fang [26] investigated the antimicrobial activity of rice vinegar against *E. coli* O157:H7 in iceberg lettuce. *E. coli* (10^7 cfu/g) was inoculated on shredded iceberg lettuce and treated with commercial vinegar containing 5% acetic acid (pH 3.0) for 5 min and it was observed that there was a significant reduction in the number of pathogens, which was 3 logs of *E. coli* population at 25 ˚ C.

Elhan [27] was studied the antimicrobial activities of six different vinegars against foodborne pathogens and spoilage bacteria in salads. In this study, a salad containing lettuce, cucumber and carrot was prepared and inoculated with *E. coli* and *S. aureus*. Inoculated salads were treated with different vinegar solutions (3%) for 0, 5, 15 and 30 min. As a result, 1 log reduction was observed, and vinegar concentration and the duration of treatment were identified to be two important parameters in decontamination.

Bornemeier et al [28] also tested vinegar (acetic acid) against *S. aureus* and *L. monocytogenes*. They observed that acetic acid inhibits the growth of these two bacteria.

Lukasik et al. [29] was used 10% vinegar in strawberries inoculated with *E. coli* O157:H7, *Salmonella montevideo*, poliovirus 1, and the bacteriophages PRD1, φX174 and MS2. As a result of this study, they observed that vinegar reduced 90% of bacteria and about 95% of viruses.

These all previous studies are supporting the results we obtained in our study. In our study we observed that grape vinegar has better activity against the microorganisms tested than Halamid®, a commercial surface disinfectant, which can be used for fruit and vegetable disinfection.

Although apple cider vinegar seems to have lower activity when compared to both grape vinegar and Halamid®, the activity can also be accepted to be very promising. On the other hand, Halamid® and apple cider vinegar didn't present any activity against *C. albicans*, but grape vinegar presented quite good activity.

5. Conclusion

Since vinegar is in the GRAS (Generally Recognized As Safe) list and natural, having such a good antimicrobial activity makes it to be a good candidate for surface disinfection of fruits and vegetables at home. Further researches should be conducted to understand the mechanism of activity.

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