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ANTIBACTERIAL ACTIVITY OF DOMESTIC APPLE CIDER VINEGAR

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ABSTRACT

In recent years, interest in examining the chemical composition and pharmacological properties of apple cider vinegar in synergy with the application of natural products in the pharmaceutical, food and cosmetics industry has been growing. The apple vinegar shows a wide range of biological activities (antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, antihypertensive, immune-stimulatory, anticancer) and it has been used in traditional medicine for a long time. It consists of 8 essential amino acids (phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine), organic acids, enzymes, minerals (potassium, calcium, phosphorus, copper, grape and boron) and vitamins (provitamin C, vitamin A, E, B1, B2, B6, vitamin P and provitamin Beta-carotene). The aim of the study was to examine the antibacterial activity of traditionally produced apple cider vinegar and to determine whether it exhibits bactericidal or bacteriostatic activity. The results of the study confirmed the antibacterial activity of apple cider vinegar produced with the traditional method. Its antibacterial activity is in the range of 11.33mm to 14mm.

Key words: *bactericidal, bacteriostatic, antibacterial, therapeutic effects, vinegar.*

INTRODUCTION

Resistance of microorganisms to antimicrobial drugs is globally expanded and its not a new problem. The number of pathogens exhibiting multiple resistance to antimicrobial drugs has been increased. The World Health Organization (WHO, 2017) predicts that antimicrobial resistance could jeopardize the ability to cure many of today's curable diseases. In immunocompromised individuals and risky patients, microbial infections can lead to sepsis, which can lead to systemic inflammation and death. As a result, in recent years there is considerable interest to employ natural antimicrobials. Apple vinegar is one of the natural products for which interest in examining its chemical composition and pharmacological

properties in synergy with the application of natural products in the pharmaceutical, food and cosmetics industry has been growing in recent years.

The use of apple vinegar has been known since ancient times, both in cooking and in traditional medicine. According to some data, the use of vinegar dates back more than 10,000 years (Tan, 2005). Old Romans used apple vinegar as a refreshing beverage while some ancient nations put it into the elixir of youth and health. Hippocrates (400 years BC), the father of modern medicine, used vinegar and a mixture of vinegar and honey for various diseases, including cough and colds, but also as an elixir, a tonic that raises energy. The apple juice was used in wars as a remedy for wound healing (Chan et al., 1993.; Tan, 2005).

In traditional medicine, apple vinegar is used in many ways because it is believed to have an antimicrobial and antiseptic effect and to help fight viruses, eliminates bacteria, fungi and other microorganisms. Likewise, in traditional medicine, apple vinegar is used in chronic fatigue, headache, insomnia, sinus pains, throat irritation, rheumatic pain in joints, extended veins, eczema, burns, excessive weight, etc. (Darzi et al., 2013).

Although the use of apple vinegar for medicinal purposes has an extremely long tradition, the chemical composition and positive influence on human health has been systematically listed and scientifically described in 1958 (Folk Medicine) (Jarvis, 1985). It has been scientifically proven that apple juice maintains vitality and refreshes the body, triggers metabolism and strengthens immunity.

Bacteria and yeasts break down natural apple sugars during fermentation. During the first phase of fermentation, sugar is converted into alcohol, and in the second, further fermentation alcohol is converted into vinegar. The whole process lasts 3-4 weeks, depending on temperature (18-25⁰C). Vinegar obtained in this way retains all the nutrients that the apple has (pectin, beta-carotene, potassium) with the addition of acids and enzymes produced during fermentation. It is a natural product based on the acidic acid that gives it acid taste and which acts as a natural antiseptic and preservative. This product is rich in minerals needed by our body including magnesium, phosphorus, calcium, chloride, potassium, phosphorus, sodium, trace minerals such as copper, fluoride, as well as vitamin A, B1, B2, B6, C and E, bioflavonoids, and pectins (Del Campi et al., 2012).

Vinegar made from wild or organic apple trees has the greatest beneficial effects. Also, it must be vinegar obtained by cold apple pressing, which is naturally naturalized, with no chemical additives (Del Campi et al., 201).

Apple vinegar is used in food products and is commonly used as a preservative for fruit and vegetable salads, as a supplement to fresh salad, in preparation of mayonnaise, mustard etc. Functional nutritional properties of vinegar have been published in various scientific and professional publications, while health benefits are still being examined. (Turker, 1963; Tan, 200; Mazza and Murooka, 2009; Ou and Chang, 2009.).

It has been scientifically proven that apple vinegar maintains vitality of the organism, stimulates metabolism, strengthens immunity, cleanses blood from toxic substances and the formation of clots, protects the heart and blood vessels, helps

regulate cholesterol and blood sugar levels. Due to its disinfection and antibacterial activity apple vinegar has a good effect on digestion, stimulating it and protecting it from various disorders, especially infections. It is beneficial for inflammation of the throat, gums, hoarseness, asthma etc. Therefore, apple vinegar has antibacterial, antioxidant, anti-cancer, antifungal, antidiabetic, anti-inflammatory, antihypertensive, immune-stimulatory effects (Nishidai et al., 2000; Ogawa et al., 2000a; Kondo et al., 2001a; Shimoji et al., 2002; Sugiyama et al., 2003a; Nishikawa et al., 2001; Iriti and Faoro 2010; Fern'andez Mar et al., 2012; Ramadan and Al-Ghamdi, 2012).

The man's aspiration to natural substances and the fear of illness caused by resistant bacteria to antimicrobial medication prompted scientists to find alternatives in the field of cattle breeding. The positive effect of apple vinegar has been proven in the prevention and in treatment of animals. It has been observed that apple vinegar has a beneficial effect on the gravidity cows, it also alleviates calving, causes an increase in milkiness, as well as a more favorable chemical and bacteriological composition of milk. The use of apple juice significantly improves the fattening and potency of bulls, reduces acute and chronic mastitis, prevents inflammation of the joints, improper heart muscle work due to thermal stress, increases appetite, reduces mortality, and according to some experiments it is a justified form of antimicrobial substitution.

Microorganisms such as *E. coli*, *S. aureus*, *C. albicans* form an integral part of human and animal micro-population. They are normal inhabitants of the skin, digestive and urinary tract as long as they are in balance with the immune and homeostatic system. If balance disorder occurs these microorganism can become pathogens that cause various diseases of the blood, urinary tract, gastroenteritis, endocarditis, soft tissue infections etc. Antimicrobials used in various diseases caused by various bacterial viruses become ineffective, ie bacteria become resistant. Various studies have shown that apple vinegar can be used to inhibit the growth of pathogenic bacterial species (Avci et al., 2007; Pourmouzaffar et al., 2017; Nazio lu,et al., 2014; Chang and Fang 2007).

The aim of the paper is to examine the antibacterial activity of apple cider vinegar produced in traditional way from wild apples and to examine the type of action on the tested bacterial species.

MATERIAL AND METHODS

Material

The research was done on a sample of local apple vinegar made from wild apples harvested in the forest in the area of Ribnik municipality (Entity of Republic of Srpska, Bosnia and Herzegovina). The process of obtaining vinegar is traditional and takes place in three phases:

1. Washed apples are pressed in order to separate the fruit juice.
2. Squeezed apple juice is filtered and placed in containers covered with gauze for 20 days;

3. After that, the juice is again poured into pots that are covered with gauze for another 20
4. days and after which the juice is squeezed and poured in glass containers.

Test culture

The study of the antibacterial activity of apple vinegar was performed on four clinical isolates (*Enterobacter kobei*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Escherichia coli*) from the collection of the laboratory of bacteriology, mycology and parasitology laboratory JU Veterinary Institute of RS "Dr. Vaso Butozan. The cultures were grown in a nutrient broth and incubated 18 h at 37⁰C. Petri plates with the appropriate medium (Müeller - Hinton agar) are seeded with 0.1 mL of a bacterial suspension with a concentration of 10⁵cfu / mL.

Test method

The agar diffusion method was used on a solid sterile nutrient medium (Müeller-Hinton agar-MHA) to test the antibacterial activity of apple cider vinegar on selected bacterial cultures.

Metal cylinders of 9 mm in diameter are placed on the surface of a solid nutrient medium to which a certain pure bacterial culture has previously been sown. Using micropipette 10 µl of apple vinegar was poured into in the cylinders. The antimicrobial drug ceftriaxone was used as a control.

The principle of this method is based on the fact that the antimicrobial agent diffuses in the medium and it radially expands, with its concentration decreasing as distance from the edges of the cylinder increases. If the bacteria is susceptible to the action of the tested antimicrobial agent, it will not grow in the zone of its action. Therefore, after incubation, the zones of absence of growth are observed around the cylinder,so called the inhibition zones. The zones of growth inhibition were measured using a ruler and the sensitivity of the bacterial strain to the tested propolis solution was determined. Petri plates were incubated for 24 hours at a temperature of 37°C

Three repetitions were made for each bacterial culture and the mean value for each bacterial culture was calculated.

Type of activity

Also, the type of action of apple vinegar is determined. To see if vinegar has bactericidal or bacteriostatic activity, a small piece of agar is taken from the inhibition zone and added to the nutrient broth. Incubation was carried out 24h at 37°C. If, after incubation, there is cloudy broth, it is considered that the vinegar is bacteriostatic, while if, after incubation, the broth remains clear, the effect of vinegar is bactericidal.

RESULTS AND DISCUSSION

The antibacterial properties of domestic apple vinegar were tested on four clinical isolates *E. kobei*, *E. cloacae*, *S. aureus*, *E. coli*, and the results are shown in the graph (Chart 1).

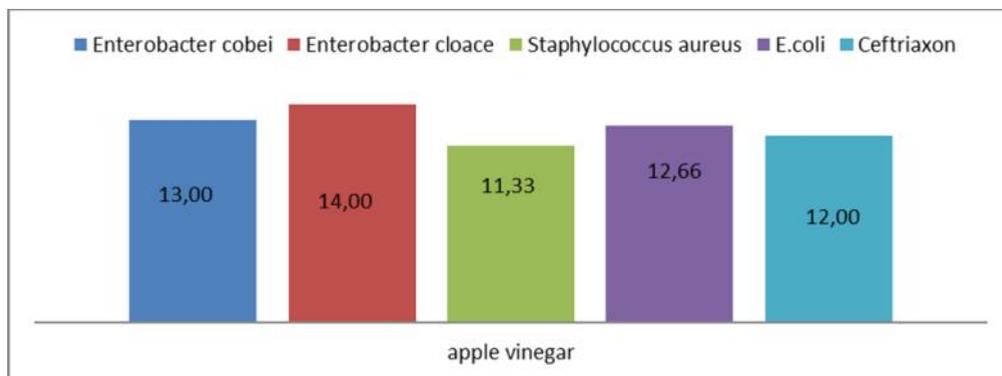


Chart 1. The antibacterial activity of apple vinegar according to the selected bacterial species (inhibition zone in mm)

As it can be seen from the chart, apple vinegar has exhibited strong antibacterial activity against the clinical isolates used in this paper. The antibacterial activity of domestic apple vinegar ranged from 11.33 to 14.00 mm, depending on the bacterial species. Various studies have suggested that apple vinegar can be used to inhibit the growth of pathogenic bacterial species in food products (Sengun and Karapinar, 2004; Wu et al 2000; Rhee et al. 2003; Chang and Fang, 2007) and that acetic acid has high inhibitory effect on *Escherichia coli* O157: H7. Likewise lactic, malic and citric acid have inhibitory effects on *E. coli*, *Salmonella typhimurium* (Yagnik et al., 2018; Entani et al., 1998; Ryu et al., 1999; Chang and Fang, 2007; Sengun and Karapinar, 2004). Apple cider vinegar acts on the bacteria by penetrating the cell wall of the bacteria and destroying their DNA and disabling their reproduction. Apple vinegar also has positive effect in gastrointestinal tract because it reduces the pH which limits the propagation of pathogenic bacteria in already consumed foods. (Yagnik et al., 2018). The inhibitory, or antibacterial effect of apple vinegar varies for each bacterial species, and the results of this work have confirmed that apple vinegar produced in a traditional way has good antibacterial potential and results are consistent with the results of other researchers who have shown that apple vinegar has multiple antibacterial potential with clinical therapeutic implications (Yagnik et al., 2018). To see if vinegar has a bactericidal or bacteriostatic effect, a small piece of agar is taken from the inhibition zone and added to the nutrient broth, and the results are shown in the chart (Chart 2.)

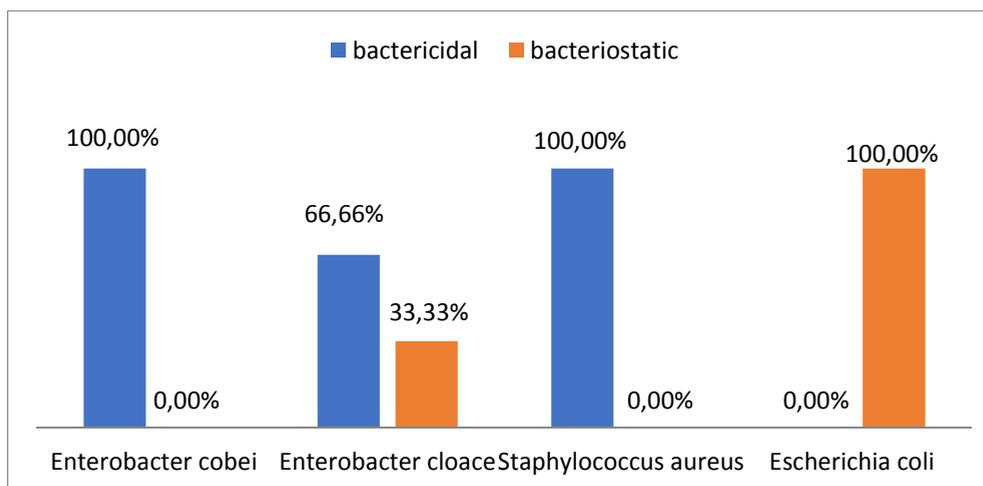


Chart 2. The type of apple vinegar activity

As it can be seen from the chart, apple vinegar displayed 100.00% bactericidal activity against *E. cobei* and *S. aureus*, and 100.00% bacteriostatic activity against *E. coli*. When it comes to *E. cloace*, 66.66% (2 reps), apple vinegar was bactericidal, and 33.33% (one repetition) it was bacteriostatic, which would be somewhat consistent with other published studies (Entani et al., 1998). Whether apple vinegar will have bactericidal or bacteriostatic activity depends to a large extent on the acetic acid concentration in the vinegar, the incubation time and the number of surviving bacteria. The bactericidal activity of apple vinegar increases with temperature. According to the results of some studies, the combination of apple vinegar with sodium chloride using the appropriate temperature proved to be very effective in preventing food poisoning. (Entani et al., 1998).

CONCLUSION

The results of the work confirmed the antibacterial activity of apple vinegar produced in the traditional way. The inhibition zone for Gram-negative bacteria ranged from 12.66 mm to 14.00 mm, while the inhibition zone for Gram-positive bacterial species was in the range of 11.33 mm. The apple vinegar had a bactericidal activity of 100.00% against *E. cobe* and *S. aureas*, and 100.00% bacteriostatic activity against *E. coli*. Due to its positive activities, its impact on improving food taste, improving digestion, and the ability to increase the body's immune response, apple cider vinegar has great potential for use in human and veterinary medicine. This is further contributed by its antimicrobial activity, which is considered a natural and acceptable replacement for antimicrobial drugs.

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