ISSN No:-2456-2165

Antibacterial Activity of Citrus Juices and Apple Vinegar on Gram Positive and Gram Negative Bacteria

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Abstract:- In the experiment we tested antibacterial activity of citrus juices and apple cider vinegar on *Staphylococcus aureus NCTC 1249,* clinical strain *Methicillin-resistant Staphylococcus aureus, Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 2523.* Antibacterial activity was tested using agar well diffusion method. Results were obtained by measuring the diameter of zone of inhibition around the wells. The highest zone of inhibition (18.5 mm) was observed around 100% concentration of apple cider vinegar on *E. faecalis.* Lemon juice of 100% concentration also had an impact on *E. faecalis* where the zone of 14 mm appeared. Zones of 15.5 mm and 13.5 mm were obtained for 100% and 75% of lemon on *S. aureus ATCC 2523* respectively. *S. aureus NCTC 1249* showed to be resistant to all concentration of all juices except for 100% concentration of apple cider vinegar where we got reduced growth. *Methicillin-resistant S. aureus* was resistant to all concentrations of apple cider vinegar and zones of 15 mm and 14 mm were present around 100% and 75% lemon juice respectively. *E. coli* showed to be resistant to all concentration of citrus juices. Apple cider vinegar concentrations of 100% and 75% slightly reduced the growth of *E. coli.* Results obtained in this experiment suggest that different concentrations of citrus juices and apple cider vinegar have a prominent antibacterial effect on gram positive bacteria and less prominent effect on gram negative bacteria as in this case *E. coli.*

Keywords:- *Antibacterial Activity; Citrus Juices; Apple Vinegar; Gram Positive Bacteria; Gram Negative Bacteria.*

I. INTRODUCTION

Antibiotic resistance is serious global problem that threatens the health of people around the world [6]. Since there are many antibiotics to which bacteria are resistant, people are increasingly starting to use traditional medicine for the treatment of infections [9, 13]. As it is known, plants and their ingredients from the early history have become an important and valuable resource which is used to treat infections and production of new drugs [2]. Currently for antibacterial purposes the most commonly used and the most accessible plants as well as their extracts in our region are lemon, grapefruit, orange and apple vinegar. The combination of apple vinegar with honey and hot water are very popular traditional medicine used for the treatment of colds [9].

Lemon belongs to the group of most essential medicinal plants. Family *Rutaceae* is recognized as origin of lemon [2]. Citrus fruits are rich in vitamin C which represents an important source for body to heal. Healthy nutritional content is present in lemon (*C. limon*) thus having significant amounts of flavanones, hesperidin and eriocitrin. Diosmin represents one of the main flavonoid ingredients of the juice as lemon is rich in flavones. Within its composition diosmetin 6,8-di-C-glucoside and apigenin di-C-glucoside are also found [7].

Its biological activities such as antibacterial, antifungal, anti-diabetic, anticancer and antiviral activities show how important lemon actually is. Today, antibacterial activity of citrus is assumed to have a great potential as source for pharmaceutical agents [10].

Grapefruit or *C. paradisi* juice in its composition possesses lycopene which has a function to determine the grapefruit color . In the absence of lycopene, the color is white while in its presence the color can vary between red and pink. Grapefruit juice composition is made out of flavanone naringin, naringenin, its aglycone and narirutin. White grapefruit juice has more flavonoids present than it is the case with red and pink ones. Naringin represents a component that gives the bitter taste to grapefruit [7]. Orange (C.sinensis) juice composition is made out of hesperidin, narirutin and didymin. All of these components belong to the group of flavanone-O-glycosides thus being the flavonoid part of the juice. Some amounts of 6,8-di-C-glucosyl-apigenin appeared as well [7].

Through the history vinegar product has been used in many civilizations for different purposes [3]. There have been a lot of studies conducted with the goal to identify all the benefits of it. The most important benefit for sure is its therapeutic effect which results from bioactive components contained therein such as 8 essential amino acids (phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine), enzymes, organic acids, vitamins (vitamin P, provitain beta-carotene, provitamin C,

vitamin A, E, B1, B2. B6), minerals (phosphorus, copper, calcium, potassium and boron) [4]. Antibiotic resistance as being serious global health problem encouraged the researchers to conduct many experiments in order to determine the antibacterial effect of plant extracts, citrus juices, apple vinegar and many other natural products. Results of all these in vitro studies were very impressive, lemon extracts showed antibacterial effect against *MRSA, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus pneumoniae*, [1, 2, 5, 8, 9, 10, 14].

The objective of our research is based on the comparison of antibacterial effect of citrus juices and apple vinegar available in our region to previous studies conducted in another countries.

II. MATERIALS AND METHODS

➢ *Citrus juices*

All citrus juices utilized in this experiment were purchased from one market in Bosnia and Herzegovina.

The origin of the orange is Spain, yellow grapefruit Italy, while the origin of lemon and red grapefruit is Turkey.

➢ Tested citrus juices:

- Lemon
- Orange
- Red grape
- Yellow grape

➢ *Apple vinegar*

Apple vinegar utilized in this experiment was 100% organic and purchased from organic shop BEEMED (Tuzla, Bosnia and Herzegovina).

➢ *Tested microorganisms*

Antibacterial activity of citrus juices and apple vinegar was tested on referent strains of following gram positive and gram negative bacteria:

- *Staphylococcus aureus NCTC 1249*
- *Methicillin-resistant Staphylococcus aureus*
- *Escherichia coli ATCC 25922*
- *Enterococcus faecelis ATCC 29212*
- *Staphylococcus aureus ATCC 2523*

➢ *Preparation of citrus juices and apple vinegar*

In this experiment following concentrations of citrus juices and apple vinegar were prepared:

- 100 %, 75 %, 50%, 25% lemon juice
- 100%, 75%, 50%, 25% orange juice
- 100%, 75%, 50%, 25% red grape juice
- 100%, 75%, 50%, 25% yellow grape juice
- 100%, 75%, 50%, 25% apple vinegar

Citrus juices were first squeezed from lemon, orange, red grape and yellow grape in sterile bottles. Different concentrations were made by mixing pure juices and apple vinegar with sterile water.

➢ *Agar well diffusion method*

Antibacterial activity of citrus juices and apple vinegar was tested using agar well diffusion method where the agar plates were inoculated by spreading 0.5 McFarland standard bacteria on Mueller-Hinton agar. An inoculum of wells of 6 mm in diameter were filled with 40 µl of different citrus juices and apple vinegar concentrations were introduced into the well.

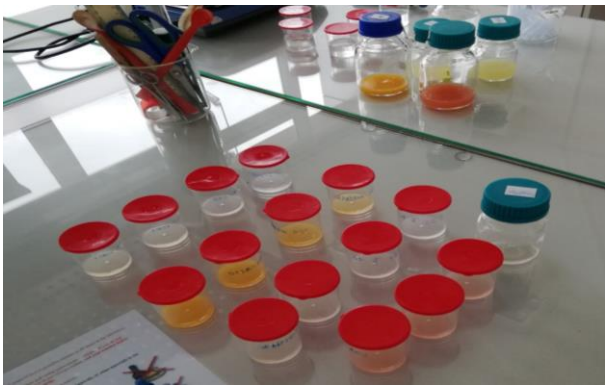

Fig 1:- Different concentrations of lemon, orange, red grape, yellow grape

III. RESULTS

The results of the effect of different lemon juice and apple vinegar concentration water solutions on the five tested referent bacterial strains is shown in Table 1.

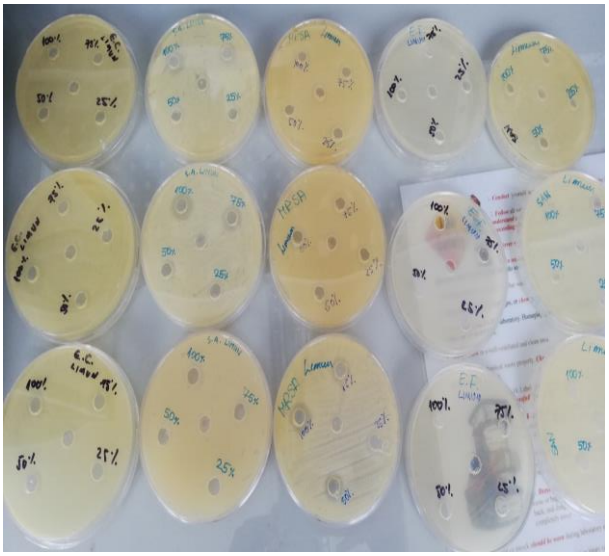

Fig 2:- Zones of inhibition of different lemon juice concentrations on five different bacterial species

ISSN No:-2456-2165

NUMBER	BACTERIAL ISOLATE	LEMON				APPLE VINEGAR			
		CONCENTRATION OF LEMON JUICE				CONCENTRATION OF APPLE VINEGAR			
		100%	75%	50%	25%	100%	75%	50%	25%
1	*Staphylococcus aureus ATCC 2523*	15,5 mm	13,5 mm	X	X	diffuse growth	diffuse growth	X	X
2	*Staphylococcus aureus NCTC 1249*	X	X	X	X	diffuse growth	X	X	X
3	*Methicillin-resistant Staphylococcus aureus*	15,0 mm	14,0 mm	X	X	X	X	X	X
4	*Enterococcus faecelis ATCC 29212*	14,0 mm	X	X	X	18,5 mm	13,0 mm	X	X
5	*Escherichia coli ATCC 25922*	X	X	X	X	slightly reduced growth	slightly reduced growth	X	X

Table 1:- Zones of inhibition of different lemon juice and apple vinegar concentrations on five different bacterial species, X designates no growth

Lemon juice concentration had an antibacterial effect on *Staphylococcus aureus NCTC 1249*and *Methicillin-resistant Staphylococcus aureus*as well as on *Enterococcus faecelis ATCC 29212*, where the highest zones of inhibition were recorded at the 100% solution.

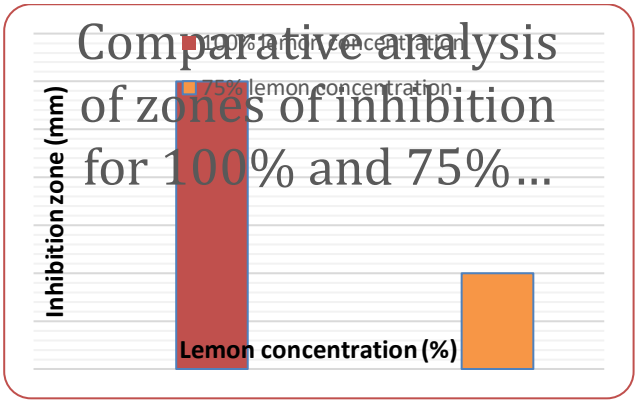

Fig 3:- Comparative analysis of zones of inhibition for 100% and 75% lemon concentration on *Staphylococcus aureus ATCC 2523*

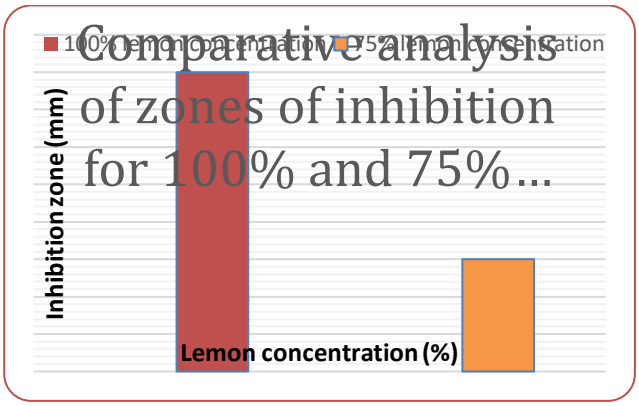

Fig 4:- Comparative analysis of zones of inhibition for 100% and 75% lemon concentration on *Methicillin-resistant Staphylococcus aureus*

Apple vinegar had an antibacterial effect only on *Enterococcus faecelis ATCC 29212*, while no zones of inhibition were recorded for other bacterial isolates. There was slightly reduced growth of *Escherichia coli ATCC 25922*while diffuse growth appeared on *Staphylococcus aureusATCC 2523*and *Staphylococcus aureus NCTC 1249.* The highest zone of inhibition around *Enterococcus faecelis ATCC 29212* appeared around the 100% solution.

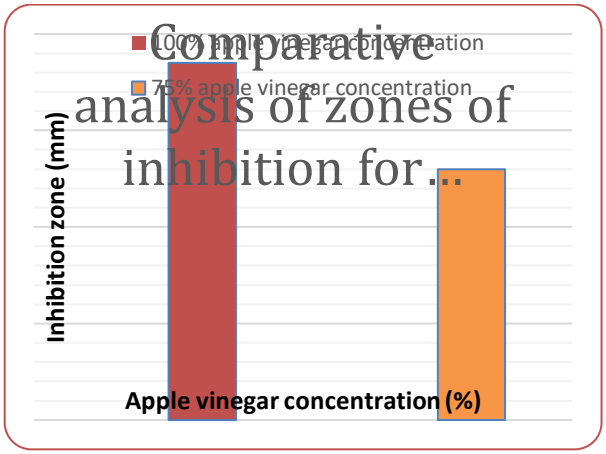

Fig 5:- Comparative analysis of zones of inhibition for 100% and 75% apple vinegar concentration on *Enterococcus faecelis ATCC 29212*

However, we tested four types of citrus juices and different concentrations. Orange, red and yellow grape with different concentration water solutions did not have any antibacterial effect on all of the tested bacterial strains.

IV.DISCUSSION

Antibiotic resistance in bacteria introduces many issues and problems in treating infections [15]. Due to this fact expanding the use of natural antibacterial substances has become one of the major issues in microbiology today [13]. From ancient times natural remedies were used for the

treatment of infections and because of that numerous studies have been conducted in order to identify natural products that have antibacterial activity [1, 2, 10, 11, 14]. Expanding the use of natural antibacterial agents is very promising due to their chemical composition and synergic antibacterial effect they have on bacteria [3, 7, 13]. Among the used natural substances that expressed antibacterial activity against gram positive and gram negative bacteria are many i.e.: citrus juices, apple vinegar, garlic and various plant extracts [1, 2, 3, 9, 10].

The aim of this study was to compare the previously reported antibacterial activities of citrus juices and apple vinegar from other studies with the ones purchased in the region of Bosnia and Herzegovina.

The study conducted by Hayes and Markovic (2002) indicated the presence of significant antimicrobial activity of lemon against *S. aureus*, *Escherichia coli*, and *methicillin-resistant S. aureus (MRSA*) [8]. Another study conducted by Hindi &Chabuck (2013) demonstrated antibacterial activity of lemon on *E. faecalis* [11]. This is in accordance to the results obtained in our study where the zones of inhibition around 100 % lemon concentration were the greatest for *S.aureus ATCC 2592*, and the lowest for *E. faecalis ATCC 29212*.

Study implemented by Kalaba et al (2019) showed that domestic apple cider vinegar has strong antibacterial effect on *S. aureus* and *E. coli* [9]. Results of our study showed only reduced growth of *S. aureus* and *E. coli*. This difference in obatined results can be explained by the fact that in the study of Kalaba et al domestic apple cider vinegar was used when compared to our study where commercially available vinegar form a local company of organic products was used.

Tawfik, Al-Haliem & Al-Ani (2010) study showed antibacterial activity of *C. limon* (lemon), *C. sinensis* (orange) and *C. paradisi* (grapefruit) against *S. aureus* [14]. In our study juice of *C. limon* showed to be more effective than juices of *C. paradisi* and *C. sinensis* because no zones of inhibition were recorded for *C. sinensis* and *C. paradisi*.

This could be explained partially by the differences in pH between *C.sinensis*, *C. paradisi* and *C. limon*. Also, charges of amino acids found within peptidoglycan could be affected by acidic pH of lemon juice [7].

Likewise, our results showed no antibacterial activity of lemon on *E. coli ATCC 25922*and *S. aureus NCTC 12493* for all tested concentrations. No antibacterial activity of *E. coli* could be explained by the cell wall composition of this gram-negative bacteria. An outer peptidoglycan layer that serves as an effective permeability barrier is present in cell wall of gram-positive bacteria, whereas outer phospholipidic membrane is consisted in Gram-negative bacteria. These differences in cell wall composition represent a reason why there is different sensitivity present between gram-positive and gram-negative bacteria [11]. *S. aureus NCTC 12493* was resistant to all concentrations of lemon juice. This can be explained by the fact that it is a methicilin-resistant strain [12].

However, *S. aureus ATCC 2592, MRSA, E. faecalis ATCC 29212*matched with above listed researches with the differences in the size of inhibition zone. Results we got for the *C. limon* extract against *S. aureus* match closely to the results of Abdullah (2009) study where the inhibition zone was 17.4 mm [1].

The limitation of our study was that a limited number of bacterial strains were tested. Also the entire fruits were used and not only the active components – hence the determination of active components within theses fruits can guide the further development of antibacterial medication.

The results of our study indicate that the concentration of the applied fruits had an effect on the zones of inhibition for *S. aureus ATCC 2592*.The antibacterial effect of lemon on *S. aureus ATCC 2592 and* apple vinegar on *E. faecalis ATCC 29212* indicates that they can be successfully used for treatment of infections.

The main conclusion drawn from our data is that the antibacterial effect of lemon extract and apple vinegar as detected in this study may partly explain the usage of these extracts as traditional medicines in Bosnia and Herzegovina against a number of infections for generations. These results give a probability of designing a potentially active antibacterial agents in the future.

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