Cumhuriyet Üniversitesi Fen Fakültesi
Fen Bilimleri Dergisi (CFD), Ciltl 36, No. 1 (2015)
ISSN: 1300-1949

Cumhuriyet University Faculty of Science
Science Journal (CSJ), Vol. 36, No. 1 (2015)
ISSN: 1300-1949

Antimicrobial Effect of Cola on Several Microorganisms

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Received: 02.10.2014; Accepted: 10.12.2014

Abstract. This study was carried out on three different brands of cola which are marketed in Turkey. Liquid portions of the collected colas were fully removed at 55 ^{o}C. Antibacterial effect spectrum was determined by Cut Plug method. Minimum Inhibitory Concentrations were determined by micro dilution method by preparing serial dilutions of cola on studied bacterial strains. It was found that cola has a strong antibacterial effect on *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Escherichia coli O: 157 H: 7, Salmonella enteritidis, Yersinia enterocolitica* bacteria and antifungal effect of cola could not identified. It was considered that cola drinks can be used for the treatment of bacterial factors and bacteria that causing food toxicity. At the end of the study, a comparison could not carried out as there was no study on the antibacterial effects of cola on various bacteria. It has been considered that cola beverages can be used for the treatment of bacterial factors and bacteria that causing food toxicity.

Keywords: Cola, Antibacterial Effect, Bacterial Diarrhea, Cut Plug Method

1. INTRODUCTION

In line with the information they got from their ancestors, people have used many methods by taking advantage of natural resources for the treatments of numerous diseases throughout history. Although medicine has developed in the recent years, this tradition based on nature still continues especially in developing communities as the resources are easily accessible and economical.

Cola was invented as syrup by J.S. Premberton who was a famous pharmacist by that time. The formula has been the world's biggest secret since the invention and has been kept safe for more than 125 years. Coca-Cola is said to be healing many diseases such as headache, weakness and morphine addiction in those years [1, 2]. In addition, cola is reported to be used for the treatment of diarrhea in remote areas of Zambia in which medical assistance is insufficient [3].

Various researches on the effect of cola on human and animal health have been carried out in recent years. These researches are mainly about the erosive and mechanic effects of cola [4]. However, there is no research on the question of whether cola has some antibacterial effects on the various bacteria or not.

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As it is known, cola has a large consumption potential all around the world. The aim of this study was to determine if cola has antibacterial effects on various bacteria. And in this research, it was intended to demonstrate experimentally whether cola can be used for the treatment of some infectious diseases.

2. MATERIALS AND METHODS

This study was made on three different brands of cola which are marketed in Erzincan (Turkey). 5x1000 ml of cola samples were collected from each brand colas were incubated at 55 ºC until liquid part evaporated. Remaining cola extract was tested by using humidity determining tool Precisa (XM60) and concentration range was determined according to the result

Antibacterial activity- Cut plug method was used in the study [5]. Each bacterial suspension as described in Table 1 was prepared as 0.5 McFarland (1×10^8 CFU/ml) in NaCl of 0,9 % from 24-hour culture in accordance with the recommendations of Clinical Laboratory Standards Institute (CLSI) [6]. Densitometer (Den-1, Latvia) was used to measure cell concentration. 1 ml of the bacteria suspension was put into the 20 ml of the prepared Mueller-Hinton Agar (Oxoid CM337); when casting temperature was reached mixing process was completed, it was left for solidifying at room temperature by pouring into the sterile petri dish. This process is performed separately for each bacterium. For retests, this amount was determined by multiplying the number of petri dishes required to be prepared. Wells in a diameter of 6 mm were opened on solidified petri dishes and 20 mg of cola extract was put into these wells. Zone diameters of the disks that were formed after 72 hours of incubation at 30 ºC for fungus and after 24 hours of incubation at 35 ºC for Mueller Hinton Agar bacteria were measured.

Determination of minimum inhibitory concentration (MIC)- Minimum inhibitory concentration was determined by micro dilution method in accordance with the procedures developed by the National Committee of Clinical Laboratory Standards [7]. Dilutions of 0,007gr/10ml, 0,015 gr/10ml, 0,03gr/10ml, 0,06gr/10ml, 0,12gr/10ml, 0,25gr/10ml, 0,5gr/10ml, 1gr/10ml, 2gr/10ml were prepared from cola extract. 50µl Mueller-Hinton Broth (Oxoid CM0405), the previously prepared bacteria suspension at 0.5 McFarland turbidity and 100µl from each cola dilution were added into each well within the 96-well micro plate. The last well was used as a negative control and cola extract was not added. After 24 hours of incubation at 37 °C, plates were shaken at 300 rpm for a period of 20 minutes in the shaker and they were read by Enzyme Linked Immunosorbent Assay Reader (Chopen)

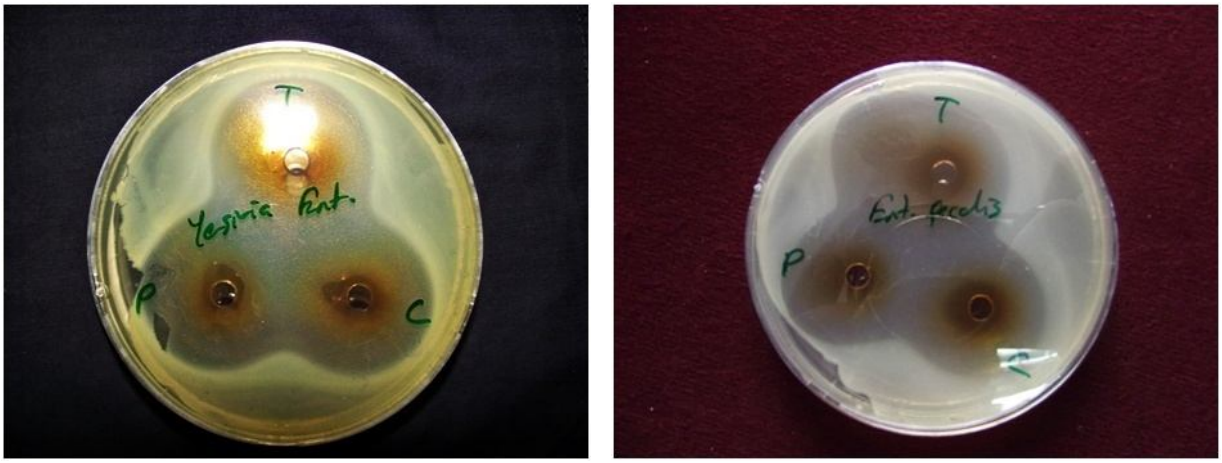
at 620 nm. Doses suppressing bacterial growth were evaluated as the minimum inhibitory concentration. Test was repeated 3 times.

Statistical analysis- Minitab 16 (Minitab Inc, State College, PA) software was used for the statistical analysis of the findings. Results were evaluated at P<0, 05 level with 2 sample t tests at a confidence interval of 95%.

3. RESULTS

The results of antibacterial effect- The research was carried out on three different cola brands and it was observed that all colas had strong antibacterial effect on *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Escherichia coli O: 157 H: 7, Salmonella enteritidis, Yersinia enterocolitica* bacteria and they had mild or weak antibacterial effect on the other bacteria used in the research. Antifungal effect of cola was not observed (Table 1, Fig. 1).

Concentration range of the colas that are ready to be consumed- It was determined that colas from three different brands had active ingredient between 9.5% - 10%.



(a) (b)

Fig. 1. Antibacterial analysis image of *Yersinia enterocolitica* (a), antibacterial analysis image of *Enterococcus faecalis (b)*.

Table 1. Antibacterial and antifungal effects of Cola1, Cola2, Cola3

Microorganism	Number	Positive control nistatin 10 ug (mm)	Positive control ampisilin sulbaktam 10 ug (mm)	Cola 1 (mm)	Cola 2 (mm)	Cola 3 (mm)
Aspergillus braciliensis	ATCC 16404	23	-	0	0	0
Bacillus cereus	ATCC 10876	-	18	**20**	**20**	**25**
Candida albicans	ATCC 10231	22	-	0	0	0
Enterococcus faecalis	ATCC 29212	-	30	**33**	**32**	**38**
Escherichia coli	ATCC 25922	-	22	**33**	**33**	**37**
Escherichia coli O:157 H:7	RHFS 232	-	23	**34**	**34**	**36**
Klebsiella pneumoniae	ATCC 13883	-	20	14	14	15
Listeria monocytogenes	ATCC 7644	-	24	10	10	12
Pseudomonas aeruginosa	ATCC 27853	-	14	8	8	8
Salmonella enteritidis	ATCC 13076	-	25	**38**	**38**	**39**
Staphylococcus aureus	ATCC 25923	-	29	12	12	14
Streptococcus epidermidis	ATCC 12228	-	22	14	14	15
Streptococcus pyogenes	ATCC 21599	-	20	9	12	10
Yersinia enterocolitica	ATCC 27729	-	22	**35**	**35**	**36**

Results of the minimum inhibitory concentration- For Cola1 and Cola2, *Bacillus Cereus, Escherichia coli O:157 H:7, Klebsiella pneumoniae, Yersinia enterocolitica* bacteria MIC value was 0,015 gr/10ml *Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes* bacteria MIC value was 0,03 gr/10ml *Salmonella enteritidis* bacteria MIC value was 0,06 gr/10 ml and for Cola3, *Enterococcus faecalis, Escherichia coli, Escherichia coli O:157 H:7, Klebsiella pneumoniae, Yersinia enterocolitica* bacteria MIC value was 0,015gr/10ml *Bacillus cereus* bacteria MIC value was 0,007 gr/10ml *Listeria monocytogenes, Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes* bacteria MIC value was 0,03 gr/10ml *Salmonella enteritidis* bacteria MIC value was 0,06gr/10ml (Table 2).

Statistical analysis results- No statistically significant difference was found between cola 1 and cola 2 except for *Streptococcus pyogenes* but the difference was considered to be statistically significant according to the control (ampisilin sulbaktam 10 ug) in three cola brands. In addition, the difference between Cola1 and Cola3 and between Cola2 and Cola3 was significant except for *Pseudomonas aeruginosa, Salmonella enteritidis, Streptococcus epidermidis* and *Yersinia enterocolitica* bacteria (Table 3).

Tablo 2. Minimum inhibitory concentrations of Cola1, Cola2 and Cola3.

Microorganism	Number	MIC Cola 1	MIC Cola 2	MIC Cola 3
Aspergillus braciliensis	ATCC 16404	-	-	-
Bacillus cereus	ATCC 10876	0,015 gr/10ml	0,015 gr/10ml	0,007 gr/10ml
Candida albicans	ATCC 10231	-	-	-
Enterococcus faecalis	ATCC 29212	0,03 gr/10ml	0,03 gr/10ml	0,015 gr/10ml
Escherichia coli	ATCC 25922	0,03 gr/10ml	0,03 gr/10ml	0,015 gr/10ml
Escherichia coli O:157 H:7	RHFS 232	0,015 gr/10ml	0,015 gr/10ml	0,015 gr/10ml
Klebsiella pneumoniae	ATCC 13883	0,015 gr/10ml	0,015 gr/10ml	0,015 gr/10ml
Listeria monocytogenes	ATCC 7644	0,03 gr/10ml	0,03 gr/10ml	0,03 gr/10ml
Pseudomonas aeruginosa	ATCC 27853	-	-	-
Salmonella enteritidis	ATCC 13076	0,06 gr/10ml	0,06 gr/10ml	0,06 gr/10ml
Staphylococcus aureus	ATCC 25923	0,03 gr/10ml	0,03 gr/10ml	0,03 gr/10ml
Streptococcus epidermidis	ATCC 12228	0,003 gr/10ml	0,003 gr/10ml	0,003 gr/10ml
Streptococcus pyogenes	ATCC 21599	0,03 gr/10ml	0,03 gr/10ml	0,03 gr/10ml
Yersinia enterocolitica	ATCC 27729	0,015 gr/10ml	0,015 gr/10ml	0,015 gr/10ml

Table 3. Statistical Analysis Result of Control, Cola1, Cola2 and Cola3

Microorganism	Number	Control - Cola1*	Control – Cola2*	Control - Cola3*	Cola1 – Cola2*	Cola1 – Cola3*	Cola2 - Cola3*
Aspergillus braciliensis	ATCC 16404	-	-	-	-	-	-
Bacillus cereus	ATCC 10876	**0,002***	**0,002***	**0,000***	1,000	**0,000***	**0,000***
Candida albicans	ATCC 10231	-	-	-	-	-	-
Enterococcus faecalis	ATCC 29212	**0,000***	**0,002***	**0,000***	0,056	**0,000***	**0,000***
Escherichia coli	ATCC 25922	**0,000***	**0,000***	**0,000***	1,000	**0,000***	**0,000***
Escherichia coli O:157 H:7	RHFS 232	**0,000***	**0,000***	**0,000***	1,000	**0,002***	**0,002***
Klebsiella pneumoniae	ATCC 13883	**0,000***	**0,000***	**0,000***	1,000	**0,002***	**0,002***
Listeria monocytogenes	ATCC 7644	**0,000***	**0,000***	**0,000***	1,000	**0,002***	**0,002***
Pseudomonas aeruginosa	ATCC 27853	**0,000***	**0,000***	**0,000***	1,000	1,000	1,000
Salmonella enteritidis	ATCC 13076	**0,000***	**0,000***	**0,000***	1,000	0,056	0,056
Staphylococcus aureus	ATCC 25923	**0,000***	**0,000***	**0,000***	1,000	**0,002***	**0,002***
Streptococcus epidermidis	ATCC 12228	**0,000***	**0,000***	**0,000***	1,000	0,056	0,056
Streptococcus pyogenes	ATCC 21599	**0,000***	**0,000***	**0,000***	**0,000***	**0,002***	**0,002***
Yersinia enterocolitica	ATCC 27729	**0,000***	**0,000***	**0,000***	1,000	0,056	0,056

*P<0.05 positive and moderately significant relationship control-cola1, control-cola2, control-cola3, cola1-cola2, cola1-cola3 and cola2-cola3.

4. DISCUSSION

Cola and cola beverages have become the most consumed beverage in the world in decades. Therefore, many studies have been carried out on cola. In addition to the researches showing harmful effects of cola, there are also studies showing the therapeutic use of this beverage.

It was reported that Hypokalemic myopathies have developed as a result of excessive cola consumption in Appel's and Rice's studies [8, 9]. There are many causes of hypokalemia. One of them is that cola contains too much sugar (11gr/dl) and insufficient amount of potassium. Osmotic diuresis develops as a result of uncontrolled consumption of cola and inadequate intake of potassium with the diet leads to lack of potassium. Another reason of this is the caffeine (97 mg/L) concentration in cola. Caffeine has antagonist effects on the adenosine receptors. Effect of adenosine receptors in many organs including kidney cause disposal of potassium through urinary tract and hypokalemia develops. It was reported that an Australian farmer had a sudden muscular atrophy on March 2002 and it was reported that the reason for this muscular atrophy was excessive cola consumption (4L/Day) [10].

Another study was carried out on the carcinogenic effect of cola. In this study, cola was given to rats in various age groups daily and the effects were evaluated histopathologically. As a result of the experiment which lasted for 104 weeks, malignant mammary tumors were detected significantly in females. In addition, pancreatic exocrine adenomas were detected both in males and females [11].

Besides, it has been reported that cola has negative effects in mouth and on teeth due to erosive effects caused by the regular consumption [12, 13, 14].

When examined studies on the use of cola for treatment purposes are analyzed, we see that [15], managed to treat gastric diospyrobezoars by endoscopic cola injection method in a study. Phytobezoar was also treated successfully with cola in another study [16].

There was no study on the antibacterial activity of cola in related literature. In this study, antibacterial spectrum of cola was determined and it was found out that cola has a strong antibacterial effect especially on *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Escherichia coli O:157 H:7, Salmonella enteritidis, Yersinia enterocolitica* bacteria and it has a weak antibacterial effect on the other bacteria used in this study. Antifungal effect of cola could not been identified.

Intestines are sterile at birth. More than 500 individual specific species and 10^4 bacteria form bacterial flora according to the mode of delivery, genetic features, environmental contact and nutrition type [17, 18]. These commensal bacteria provide significant benefits on nutrition, angiogenesis, and

mucosal immunity. Intestinal bacteria are also involved in bile salt metabolism, lipid hydrolysis, fragmentation of proteins into peptides and amino acids and vitamin synthesis [18, 19].

Antibacterial effects of uncontrolled consumption of cola on the intestinal flora should also be noted. It should not be ignored that uncontrolled consumption of cola may cause immune system failures, vitamin deficiencies, and digestive problems. In addition, it is thought that cola can be used for the treatment of bacterial factors and on bacterial diarrhea causing food toxicity.

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