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Bactericidal Effect Of Common Chemicals On The Growth Of Bacterial Pathogens

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Abstract:

The following study was designed and conducted to investigate the bactericidal effect of common chemical, extensively used in household and laboratories which is responsible to contaminate the environment and affect the normal flora of human. The test shows the excellent bactericidal effect of citric acid and acetic acid with the significant level was from P < 0.05.Salmonella typhi a causative agent of typhoid fever also restrain effectively with citric acid and acetic acid.Boric acid,a major active compound of many pesticides has show little effect on the growth of S.aureus and E.coli.Hence,a non significant effect of sodium hydroxide,sodium bicarbonate and boric acid effects observed against all four strain of bacteria.with the comparision of standard antibiotics,namely,Cefixime and levoflaxacin,it is evaluated that the citric acid and acetic acid have high efficiency against S.aureus,B. Subtilis, S. typhi, and E. coli then standard antibiotics.It was concluded that among six commonly used chemical citric acid and acetic acid are highly effective,cheap,efficient,safe and environmentally friendly product, therefore, seems to be used in pharmaceutical industries or food industries by using it a suitable and cost effective antiseptic agent and disinfectant, but further clinical research necessary.

KEYWORDS:

Bactericidal, boric acid, environmental friendly, citric acid, acetic acid

INTRODUCTION:

Emerging of new strains of bacteria and their resistant power of many antibiotics resulting a more serious health issues and introducing a new infectious disease in a community [1] *Escherichia coli, Staphylococcus aureus, Bacillus Subtilis* and *Salmonella typhi* consider as an opportunistic pathogen. Bacillus Subtilis cause several food poisoning problems [2]. *Escherichia coli* is enlisted as a normal flora of intestinal track of mammalian, including the human and bird. It is usually used as an indicator of fecal pollution in dairy products, food and water.Although *E. coli* is harmless bacteria, but few are pathogenic cause intestinal disease in man including diarrhea and other food related illness when contaminated food and water are consumed [3,4].

Staphylococcus aureus is the included as a dominant pathogenic bacteria causing of nosocomial infection and nosocomial pneumonia. In opportunistic conditions *Staphylococcus* aureus cause lifetreating illness in immunocompromised population. They also have the ability to cause postoperative wound infection skin syndrome,Toxic shock syndrome, food poisoning,mastitic and urinary tract infection [5-7]. Salmonella typhi is a leading cause typhoid fever [8]. It is a transferable bacteria which are transferred by the mean of oral fecal route [9]. *S. typhi* can cause lifelong illness in human populations ,in which majority are colonizing the gall bladder [10],and also responsible for the destruction of intestinal leading hemorrhage. *Salmonella* prevails for a long time in the environment, even it at present in Eggs and poultry food [5].

In Developing countries, the total rate of chronic and opportunities infection is high [11]. Hence it is, necessary to look for more effective, safer and less toxic products and compounds for the treatment of new emerging disease in the society that's why the main object of this study is to exam the commonly used chemical on the opportunistic pathogens.

MATERIALS AND METHODOLOGY

Chemical Use

Six types of commonly used chemical namely Acetic acid of 0.75 M, Calcium hydroxide of 0.02 M, Sodium bicarbonate of 0.1 mM, and Sodium hydroxide of 1.25 M, Boric acid of 0.6 M and Citric acid of 0.5 M were prepared in the laboratory of Institute of Environmental Studies, University of Karachi.

Bacterial Strains

Four strains of bacteria were used in this study, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli* were obtained from the Institute of Environmental Studies, University of Karachi. These strains were maintained on a nutrient broth at 4 °C. Strains were then transferred to fresh nutrient broth and incubate at 37°C for 24 hours before use.

Antibacterial assay

The antimicrobial activities of six commonly used chemicals were tested using the paper disk diffusion method [12].Filter paper disc of 6 mm was used for this studies soaking with 0.025 ml of each acid, placed on inoculated nutrient agar plates. All plates were incubated at 37°C for 24 hours. The antibacterial effectiveness of each chemical was determined by measuring the diameter of the zone of inhibition formed around the discs. For the sake of accuracy the test was done in triplicate.

Statistical analysis

Given all the data were statistical analyses and expressed as mean ± standard deviation, a further calculation of T-test and the level of significance was from $P < 0.05$ for Acetic acid,citric acid and sodium hydroxide.As for the data and graphs they were analysis using Microsoft® Office Excel 2007.

RESULT AND DISCUSSION

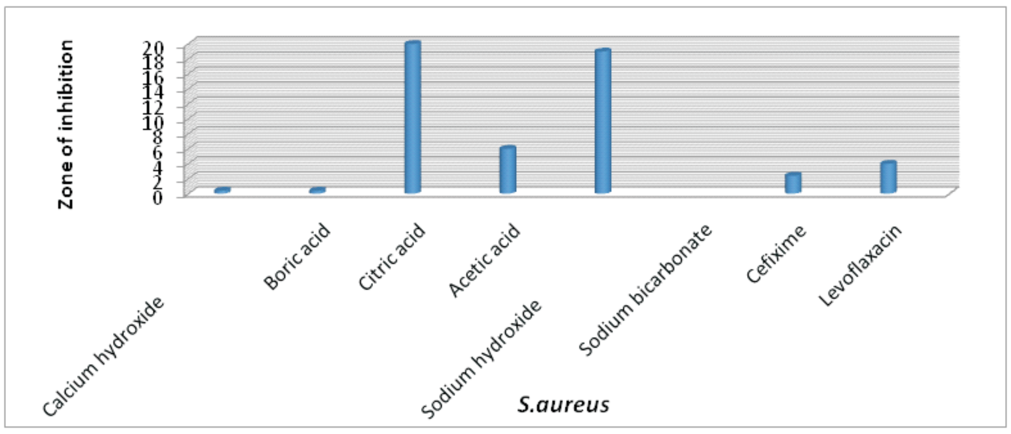
A preliminary study was conducted to determine the inhibitory power of organic acids against four strains of bacteria, namely, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Escherichia coli. Escherichia coli* and *Staphylococcus aureus* are most leading pathogens with high cross contamination rate [13].In table 1 it is clearly stated that all the treatments have some lethal effect on bacteria.However,boric acid, an integrated active part of all pesticide products including wood preservative,fungicides,algaecides and insecticides [14] have no effect on *B.subtilis , E.coli and S. typhi*.In our studies *Escherichia coli* show no growth inhibition effect against Calcium hydroxide, Sodium hydroxide and Sodium bicarbonate, but on other hands it show excellent inhibition against Citric acid (16 mm) and Acetic acid (14mm).According to Montville and Matthews *E.coli* have acid tolerance and can grow at a pH as low as 4-4.5.Hence we can say that the resistivity of *E.coli* against Calcium hydroxide, Sodium hydroxide and Sodium bicarbonate is due to their low pH growth ability. This acid tolerance can induced other stress response in *E.coli* such as radiation, heat and antimicrobial tolerance [15].The only one bacteria on this study *Staphylococcus aureus* was inhibited effectively with Sodium hydroxide (19 mm), particularly this value is much higher than the standard antibiotic zone, elaborated in graph 1.The antibacterial activity of sodium hydroxide was also fair against S.typhi (3 mm) which was higher than the cefixime drug (0.8 mm),the overall level of significance of sodium hydroxide was from $P < 0.05$.There was no growth retardation seen to the culture of Staphylococcus aureus treated with Sodium bicarbonate.

Citric acid and acetic acid, consider a most acidic chemical in nature showed excellent results among all treated chemicals with the level of significance was from $P < 0.05$. Our study supports the research of Ryssel *et al* [16] who showed excellent bactericidal activity of acetic acid, particularly with Gram-negative bacteria. It has been stated that most of the microorganisms grow best at pH of about 7.0 [13], therefore the alternation of pH in our studies by treating chemicals, especially acidic nature chemical citric acid and acetic acid provide most promising results. In past years, the various researchers study has been done to prove the antibacterial effect of organic acids on different types of pathogenic bacteria [17-19]. Raftari *et al* in his studied used acetic acid to reduce the contamination in meat and found the significant reduction of *S. aureus* and *E. coli* after the treatment with acetic acid [20]. These two chemicals showed higher activities against all strains of bacteria when compared with standard reference antibiotics Cefixime and levoflaxacin shoes in graph 1-4.However; the use of this type of chemicals without any precaution can also affect the normal flora of our body.

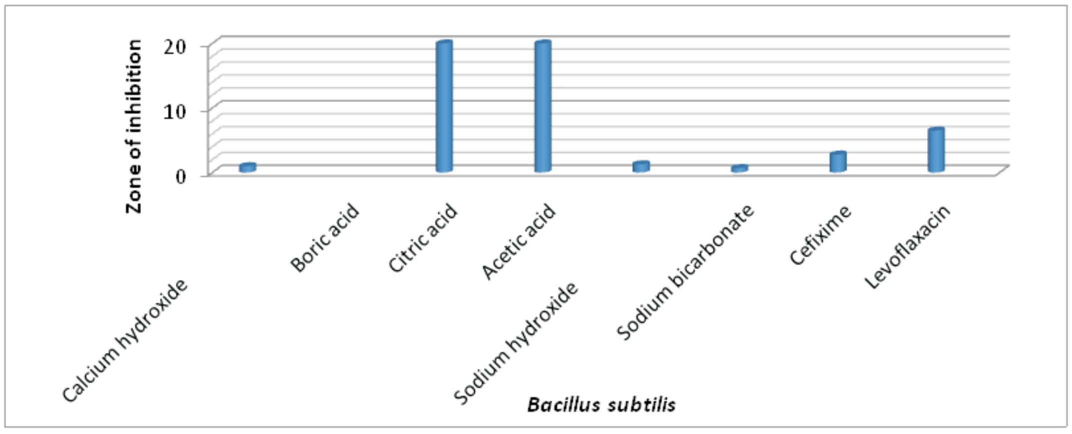
Table 1: Zone of inhibition of organic chemical against different strains of bacteria

	Microorganisms			
Common Chemical	*S .aureus*	*B.subtilis*	*S. typhi*	*E.coli*
Calcium hydroxide	0.4±0.08	1±0.4	1.2±0.06	__
Boric acid	0.4±0.2	__	__	0.5±0.2
Citric acid	20±0.8	20±0.5	15±0.6	16±0.3
Acetic acid	6 ± 0.2	20 ± 1	7 ± 0.3	14±1
Sodium hydroxide	19±0.5	1.3±0.1	3±0.3	__
Sodium bicarbonate	__	0.7±0.1	2±0.06	__
Cefixime	2.4 ±0. 3	2.8 ±0. 1	0.8±0.5	1.5±0.2
Levoflaxacin	4±0.5	6.5±0.7	6.9 ±0. 7	4.5±0.3

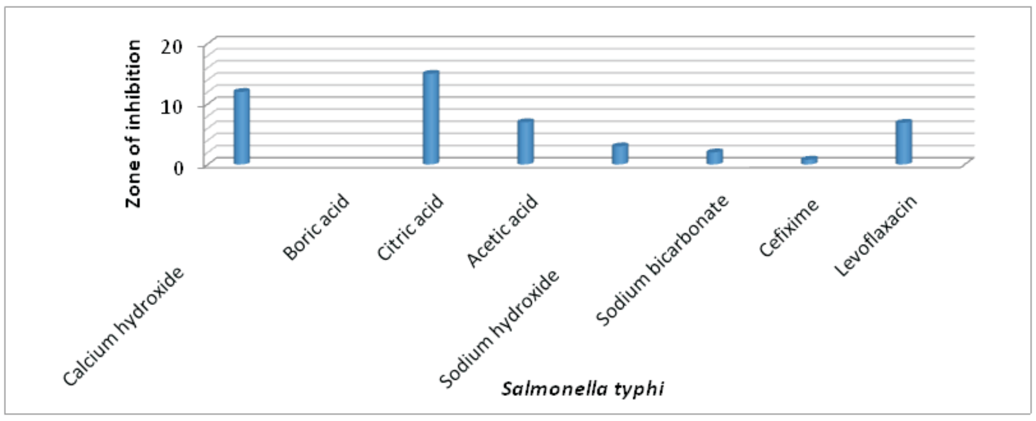
-- = No effect on bacteria growth
*All the zone of inhibition measure in mm



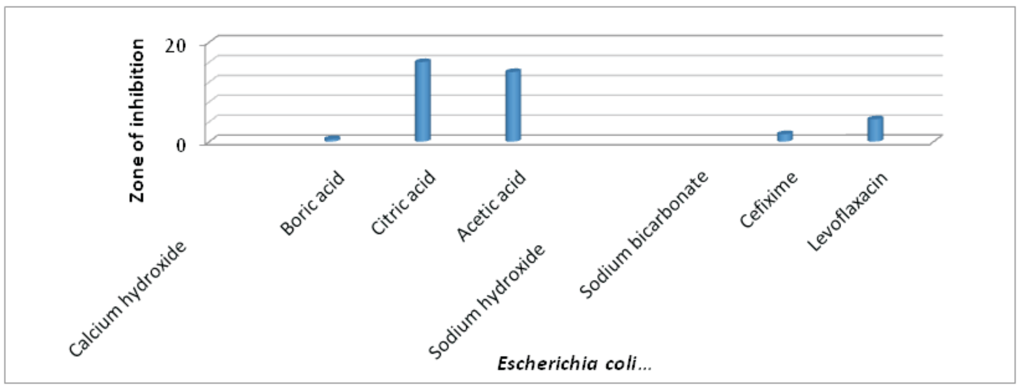
GRAPH 1: Shows the zone of inhibition of six organic chemicals and standard antibiotics on the *S.aureus*



Graph 2: Illustration of inhibition potential of organic chemicals with the comparison of reference drugs of *Bacillus subtilis* culture.



Graph 3: Explaining the effect of chemicals and reference antibiotic on the growth of pathogenic bacteria



Graph 4: Graphically representation reveals the power of chemicals and their comparison on the growth mechanism of *E.coli*

CONCLUSION:

It is concluded from this preliminary study date these understudy chemicals, especially citric acid and acetic acid can apply effective for the control of disease spread or for the new intervention of environmental friendly product. Therefore,it can be recommended to be used in pharmaceutical industries or food industries by using it as a suitable and cost effective antiseptic agent and disinfectant, but more further clinical research necessary.

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