IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 7 Ser. II (July 2022), PP 20-22
www.iosrjournals.org

Antimicrobial Activity of Undistilled Cow Urine on Prevalent Water Borne Pathogens

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Abstract
Disinfection of water is generally accomplished by adding some form of chlorine, either as bubbled gas or aqueous solution of bleaching powders. In both cases chlorine behaves as a toxic and corrosive gas, for both the handlers as well as the consumers. It causes skin burns, eye irritations and gastro intestinal problems. Moreover, the commercial production of hypochlorite is tedious, expensive and an established environmental hazard. This contribution is in the initial stage of a two pronged study, first, on the composition and effective concentration of an organic biocide using raw cow urine as one of the ingredients and second, the effect of different concentrations of the formulation on spores of Giardia lamblia, Cryptosporidium, Streptococcus, Escherichia coli, Clostridium perfringens and some common bacterial strains found in swimming pools.

Date of Submission: 08-07-2022 Date of Acceptance: 22-07-2022

I. Introduction

Before the nineteenth century, water in primitive lands needed no disinfection. But now in the developed world, there is virtually no clean water or soil left. According to a 2007 World Health Organization report, 1.1 billion people lack access to an improved drinking water supply, 88% of the 4 billion annual cases of diarrhea are attributed to unsafe water and 1.8 million people die from diarrhea diseases each year. [1]

Disinfection of water is accomplished by adding disinfectants which are generally chemicals in some form of chlorine either as bubbled gas or as hypochlorite (bleaching powder). In both cases chlorine behaves as a toxic and corrosive gas. A major drawback of chlorine disinfection is that the technology employed for chlorination and the commercial production of hypochlorite is expensive, tedious and a proven environmental hazard. [2]. In 1970s, Environmental Protection Agency (EPA) determined that chlorine from any source reacts with natural organic compounds of contaminated water (which are in high percentage) to form potentially harmful chemical by-products like Trihalomethanes (THMs) and Haloacetic acids (HAAs), both of which are carcinogenic in addition to small amounts of chloroform. Unless free chlorine is measured, disinfection by chlorination can not be guaranteed.

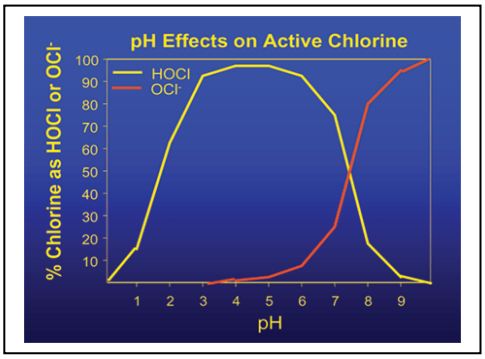
Bleach is a strong oxidizer and products of the oxidation reactions are corrosive. [3] It burns skin and cause eye damage. Household bleach and pool chlorinator solutions are typically stabilized by a significant concentration of lye as part of the manufacturing reaction and human skin contact produces caustic irritation or burns due to saponification of skin oils. [4]

Chlorine treatment via hypochlorite is susceptible to variations in the pH. At lower pH, undissociated hypochlorous acid dominates the reaction, while at higher pH (pH>8), HOCl tends to dissociate into hydrogen and chlorite ions, which are less effective as a disinfectant as compared to HOCl.

$Cl2 + H2O <—> HOCl + HCl$ (low pH)

$HOCI <—> H+ + OCl-$ (high pH)

As a result, chlorine effectiveness drops off when the pH is high.



Although chlorine is effective in killing microbes and bacteria, it still has limited effectiveness against protozoa that form cysts in water (Giardia lamblia and Cryptosporidium, both of which are pathogenic). [5] The effect of the organic biocide on these two pathogens and clostridium perfringens forms the next stage of study.

The standards for drinking water quality are typically set by governments or by international standards for the minimum and maximum concentrations of contaminants allowed for water of particular areas. This research will focus on how to achieve these typical standards of water quality by using an organic biocide prepared typically from undistilled cow's urine and provide an alternative to chlorination of water.

II. Materials and Method

Collection of water samples: In the first stage of work, three water samples were collected from three different water bodies. Two water samples were collected from two separate swimming pools (one from Aligarh Club and second from Aligarh Muslim University swimming pool), situated in different localities and maintained by different bodies. Third sample of water was collected from the municipal water supply of Aligarh.
Water samples from the swimming pools were collected in sterile glass bottles (with glass stoppers) of 250 ml capacity containing 0.5 ml of 5 % sodium thiosulphate. The bottle was sterilized along with the thiosulphate. The water was collected from a depth of one foot (approx.).It was mechanically filtered through Whatmann paper to remove suspended impurities.
The water from municipality was also collected in sterile glass bottles (with glass stoppers) of 250 ml capacity. The municipal water tap was flamed and the water allowed to run for two minutes before collection. It was also mechanically filtered as above.

Collection of urine samples: 5 liters of cow urine was collected from a 'Gowshala' in Surya Vihar, Ghaziabad and another 5 liters was collected from the cow of a local neighbor in Janakpuri colony Aligarh. The samples were collected in sterile glass jars with stoppers and stored at 40C in refrigerator. The urine samples were mechanically filtered through Whatmann paper to remove suspended impurities.

Collection of cow dung samples: Approximately 5 kilograms of fresh local cow dung was collected and kept in plastic tubs. It was covered by paper sheets to prevent infestation by flies.

Preparation of Bijamrit: The formulation named as 'Bijamrit' was prepared using twenty liters of distilled water, five kg of local cow dung and five liters of local cow urine, fifty grams slaked lime and a handful of farm soil. The local cow dung was tied in a cloth by tape and was hung in twenty liters of water in a barrel for more than twelve hours. Fifty grams of slaked lime was added to one liter of this impregnated water in barrel and was left overnight. The next morning, the cloth bundle containing the cow dung was squeezed in water in barrel, so that all essence of cow dung passes into the water. Then a handful of farm soil, 5 liter cow urine or human urine and one liter of the stabilized lime water was added to the mixture and stirred well.

Test organisms: Bacterial and fungal cultures of Escherichia coli and Streptococcus were prepared at the laboratory in two separate experiments and identified by a bacteriologist.

Microbial Analysis Organisms: The water analysis for microbes started within six hours of sample collection for all three samples. The Standard Plate Count method was employed to culture the microbes. The colonies of E.coli were developed on Nutrient agar, MacConkey broth, M-Endo broth, those of streptococci on Azide Dextrose Broth and incubated at 37^0C for 48 hours. The results were expressed as Number of Colonies / ml for E.coli and streptococci.

pH Analysis: pH analysis of the water, cow urine and the bijamrit was conducted using digital pH meter.

Work Analysis: The sensitivity of these microbes was determined against sodium hypochlorite and the Bijamrit and Mean and Standard deviation were calculated to facilitate the comparison of the data.

III. Results and Discussions

The result of the biocidal property of Bijamrit show that the activity of undistilled cow urine preparation was better than the values that have been reported for cow urine distillate and photo activated urine distillate. [6] The reason may be the availability of certain volatile and nonvolatile components present in undistilled cow urine. [7] The activity of bijamrit preparations was comparable to that shown by hypochlorite solutions. [6]

The mean zone of inhibition (mm) for the hypochlorite solution and Bijamrit was comparable for Escherichia coli and faecal coliform. When the quantity of slaked lime was increased in the Bijamrit the mean zone of inhibition (mm) for Bijamrit exceeded that for the hypochlorite. The increase in efficacy might be due to the individual activity of the unreacted calcium hydroxide in the organic preparation. [8]

The mean zone of inhibition (mm) in case of streptococcus was less for bijamrit as compared to hypochlorite. Further studies will be conducted after altering the percentage and the composition of the ingredients of bijamrit for improving the antimicrobial activity on streptococcus as well as prevalent protozoa and bacteria.

Gram positive organisms are more sensitive than gram negative organisms. This may due to the difference in the cell wall structure between the two strains. [9] This predicts that Clostridium should be inhibited by the addition of biocide. The concentrations and percentage compositions need to be determined.

IV. Conclusion

Numerous antibiotics, pesticides and disinfectants have been formulated and copiously administered to the environment in a raging war to control microbial infections and diseases. As nature works, new strains of virus and bacteria emerge every year and old strains become extinct. Microbes have developed resistance against many broad-spectrum antibiotics, pesticides and disinfectants. To combat the problem of microbial infections and resistance many natural products like cow urine, possessing antimicrobial properties, have been explored. The present study was undertaken to determine antibacterial and, antifungal activity of raw cow urine in combination with cow dung, soil, slaked lime and distilled water. The antibacterial activity was tested against Gram positive and Gram negative bacteria. Further studies on the exact composition and *in vitro* experiments are to be carried out. The final aim is to reveal the antimicrobial activity *in vivo*.

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Viniti Gupta, et. al. "Antimicrobial Activity of Undistilled Cow Urine on Prevalent Water Borne Pathogens." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 15(07), (2022): pp 20-22.