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# Minimum Inhibitory Concentration (MIC) Of various synthetic and natural antimicrobial agents using *E coli* screened from VIT sewage treatment plant*

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**Abstract:** The Minimum Inhibitory Concentration (MIC) Test is an important evaluating tool in the field of microbiology. It gives us an idea of the microbial activity assessment in any source sample containing microbes. Validating the effectiveness of disinfection and decontamination is a vital and often challenging task.[1] In clinical and laboratory environments, presence of sterile conditions is a compulsion and various disinfectants are used to sterilize the surroundings from bacteria, fungi and other microorganisms. The broth dilution and agar diffusion methods of MIC Test tell us about the efficiency of the disinfectant under use.[1,2] By subjecting a given disinfectant sample to the MIC Test, we can find out its efficiency in lysing the microorganisms present in the inoculums. Thus two or more disinfectants can be compared also at the same time to test their lysing capacity under normal conditions. The MIC Test also helps to choose the more economic disinfectant from a given batch of disinfectants. The results obtained from the MIC Test can also be used to prepare a new anti microbial agent which has greater efficiency in lysing the microorganisms and is economic financially. Minimum Inhibitory Concentration (MIC) of various synthetic like chloramphenical, chloroxylenol and 10% hydrochloric acid with butyl oleylamine in aqueous solution), and natural antimicrobial agents like Sample C (Grass extract) and Sample D (Grass+Neem extract) using *E coli* screened from VIT sewage treatment plant will be discussed.

## 1. Introduction

In pharmaceutical research, Minimum Inhibitory Concentration (MIC) is the least concentration of an antimicrobial agent that inhibits the growth of a microorganism after a given period of incubation.[3] In Diagnostic laboratories MIC helps in confirmation of resistance of microorganisms to an agent and also to monitor the activity of new antimicrobial agents. Determination of MIC is done using dilution method which is also a reference for various other methods like disc diffusion. For comparative testing of new agents minimum inhibitory concentration test is very effective. There are three conditions under which MIC test is favoured first in case of equivocal results in disk tests second when a more accurate result is required and third for tests when disk tests may not give appropriate and reliable results.[2,4]

The objective of broth and agar dilution methods is to determine the minimum concentration of the assayed antimicrobial agent that inhibits the growth of the bacterium under specified conditions. MIC values are

used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents.[5]

Results are interpreted with the help of UV Spectrophotometric techniques. Absorbance at 540 nm in the UV Spectrometer tells us about the microbial activity after overnight incubation. Higher the absorbance more the microbial activity.

## 2. Experimental

### 2.1 Preparation of Nutrient Media

2.8 grams of Nutrient Media was weighed.100 mL of distilled water was taken in a conical flask and to the distilled water, the weighed nutrient media was powder was added. The conical flask was stirred thoroughly. The media was kept for sterilization in the autoclave along with the glassware.[5,6]

**Fig 1.nutrient media after sterilisation**

### 2.2 Preparations of disinfectant solutions:

Sample A (4.8% chloroxylenol )was taken in a test tube was taken. It was diluted with distilled water and stored in glass test tubes. The dilutions taken into consideration were 1%, 5%, 10% and 15% in 10mL total volume. Same set of solutions were made for Sample B (10% hydrochloric acid with butyl oleylamine in aqueous solution), Sample C (Grass extract) and Sample D (Grass+Neem extract).

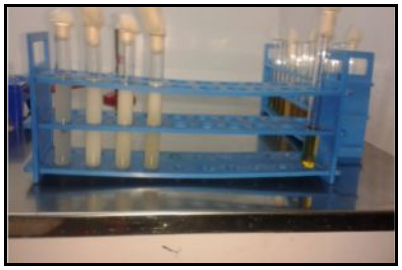

**Fig 2.Sample solutions of varying concentrations**

### 2.3 Inoculation of bacterial culture:

5mL of the prepared and sterilized nutrient media was poured into 20 test tubes. To this, 1 mL of bacterial culture (E.coli ) were inoculated. A blank solution or control was prepared containing only the nutrient media i.e 5mL. The entire procedure was conducted in a sterilized laminar airflow chamber.[6]

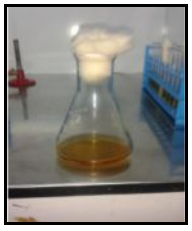

**Fig 3. Bacterial culture.**

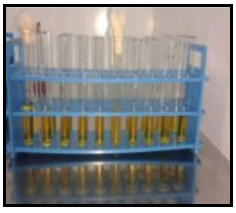

**Fig 4.Test tubes with nutrient media and bacterial culture.**

### 4.5 Addition of disinfectant solution:

To the nutrient media containing the inoculums, 1mL of the 1% solution from Sample A was added at 0 minutes. Similarly, 1mL from 5%, 10% and 15% were added to the test tubes. The procedure was repeated at intervals of 15 minutes i.e. 15 minutes, 30 minutes, 45 minutes and 60 minutes. This was done for all the concentrations of Sample A. The above procedure was carried out for Sample B, Sample C and Sample D. To avoid contamination, the procedure was carried out in a sterilized laminar air flow chamber. After carrying out the above procedure, the test tubes containing the inoculums and the disinfectant were placed in the incubator overnight at 35°C. After incubation, the test tubes were taken out of the incubator. They were then subjected to UV Spectrophotometry at 540 nm.The test tube containing the control served as a reference. The UV Spectrophotometer readings were then recorded and then analysed.[6,3,1]

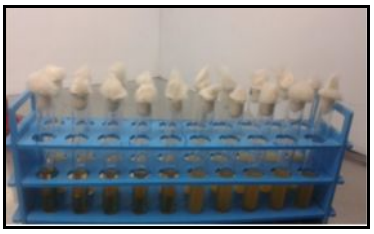

**Fig 5 Blank/Control solution. Fig 6 Test tubes containing nutrient media with inoculum and disinfectant.**

## 3. Results and Discussion

After incubating overnight at 35°C and subjecting it to UV Spectrophotometry, the following results were observed for Sample A, Sample B, Sample C and Sample D.

**Table 1. Sample A**

| concentration | 0 min | 15 mins | 30 mins | 45 mins | 60mins |
|---|---|---|---|---|---|
| 1% | -0.242 | -0.237 | -0.221 | -0.186 | - 0.200 |
| 5% | 1.848 | 1.790 | 1.703 | 1.685 | 1.646 |
| 10% | 2.727 | 2.603 | 2.505 | 2.221 | 2.503 |
| 15% | 2.893 | 2.778 | 2.750 | 2.710 | 2.706 |

**Table 1. Sample B**

| concentration | 0 min | 15 mins | 30 mins | 45 mins | 60mins |
|---|---|---|---|---|---|
| 1% | -0.171 | -0.284 | 0.314 | 0.217 | 0.109 |
| 5% | -0.315 | -0.269 | -0.309 | -0.0032 | -0.300 |
| 10% | -0.298 | -0.277 | -0.285 | -0.246 | -0.279 |
| 15% | -0.312 | -0.233 | -0.307 | 0.163 | -0.305 |

**Table 3. Sample C**

| concentration | 0 min | 15 mins | 30 mins | 45 mins | 60mins |
|---|---|---|---|---|---|
| 1% | 1.110 | 0.937 | 0.695 | 0.850 | 0.845 |
| 5% | 1.475 | 1.300 | 1.550 | 1.453 | 1.450 |
| 10% | 1.626 | 0.543 | 0.517 | 1.500 | 1.470 |
| 15% | 1.658 | 1.645 | 1.553 | 1.558 | 1.680 |

**Table 4. Sample D**

| concentration | 0 min | 15 mins | 30 mins | 45 mins | 60mins |
|---|---|---|---|---|---|
| 1% | 0.705 | 0.750 | 0.930 | 1.072 | 1.116 |
| 5% | 0.980 | 1.190 | 1.350 | 0.930 | 1.290 |
| 10% | 1.470 | 1.338 | 1.530 | 1.400 | 1.540 |
| 15% | 1.725 | 1.675 | 1.610 | 1.630 | 1.690 |

The main aim of the MIC Test is to check microbial activity, the absorbance of the samples containing the inoculums and disinfectant.The MIC Test tells us about the efficiency of the given anti microbial agent. Thus, a subsequent rise or drop in the absorbance values tells us about the efficiency of the given anti microbial agents.[7] In Sample A, 4.8% chloroxylenol , the 1% concentration which was taken under consideration showed negative values of absorbance initially. The values obtained afterwards for 1%,5%,10% and 15% gave a decreasing trend of the absorbance values. The decrease in the values of absorbance can lead to the fact that microbial activity was diminished due to the rise in concentration of the sample.[8,9]

In Sample B, 10% hydrochloric acid with butyl oleylamine in aqueous solution, however the absorbance values are in the negative range. This can be due to the over exposure of the disinfectant to the microbial culture leading to extremely diminished microbial activity. Also contamination while introduction of the bacterial culture to the nutrient media may lead to a change in the absorbance values. The calibration of the sample with the control may lead to the negative values. However, a decreasing trend was observed in the absorbance values leading to the fact that Sample B can be a potential anti microbial agent. In Sample C, which was a grass extract taken under our study gave positive results. The absorbance values gave a decreasing trend with increased time of incubation. The decrease in the absorbance values can conclude that Sample C can be used an antimicrobial agent. In Sample D, which was Grass and Neem extract, the absorbance values showed a significant decrease. This is due to the antimicrobial activities present in the Neem (*Azadirachta indica*) leaves. This lead to the fact that naturally available plant products Neem leaves and grass can be used as an effective and potent anti microbial agent for further research.[10.11]

## 4. Acknowledgement

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