Studies of Chemical disinfectants and their role as antimicrobials

M.Sc. THESIS

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By

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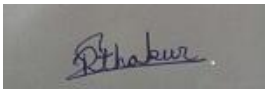
DECLARATION

I hereby declare that the work presented in the thesis report entitled "**Studies of Chemical disinfectants and their role as antimicrobials"** submitted for partial fulfilment of the requirements for the degree of Master of Science in Biotechnology at Jaypee University of Information and Technology, Waknaghat is an authentic record of my work carried out under the Supervision of Dr. Jitendraa Vashistt, Assistant Professor. This work has not been submitted elsewhere for the reward of any other degree/diploma. I am fully responsible for contents of my seminar report.



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CERTIFICATE

This is to certify that the work entiled "Studies of Chemical disinfectants and their role as antimicrobials**"** submitted by Rajeshwari Thakur in partial fulfillment for the award of the degree of **MASTER OF SCIENCE IN BIOTECHNOLGY** from the Jaypee university of Information and Technology, Waknaghat Solan has been carried out under my supervision. This work has not been submitted partially or wholly to any other institute for the award of this or any other degree or diploma.

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SUMMARY

In our current situation Disinfectant plays major role in our daily life from eating to travelling we all uses Disinfectants many times in a day. The study shows the necessity of Disinfectants in our daily life. Many people are unaware of hazardous effect that causes many types of respiratory, reproductive and many kind of diseases. The MIC results shows how some chemicals and their combination work very prominently against microorganisms. Also lacking of proper guidance causes the dangerous combination of chemicals which are very harmful. Highly effiecient with less concentration of toxicity is the main purpose of this study by checking MIC and antimicrobial property on different types of bacteria commonly found in our places. The activity of different chemicals and their effective concentrations can bring the positive changes in our environment.

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ABBREVIATIONS

D.W.	Distilled Water
^{o}C	Degree Celsius
MIC	Minimum Inhibitory Concentration
CTAB	Cetrimonium bromide
CJD	Creutzfeldt–Jakob disease
TSB	Trypticase soy broth
HSV	Herpes Simplex Virus
HIV	Human Immunodeficiency Virus
S.cerevisiae	*Saccharomyces cerevisiae*
M.Chelonae	*Mycobacterium chelonae*
CHG	*Chlorohexidine gluconate*
C.albicans	*Candida albicans*
BI	Biological Indicator
BSE	Bovine Spongiform Encephalopathy
i.e.	In essence
B. stearothermophilus	*Bacillus stearothermophilus*
E.cloacae	*Enterobacter cloacae*
S. marcescens	*Serratia marcescens*
B. subtilis	*Bacillus subtilis*
A.calcoaceticus	*Acinetobacter* calcoaceticus
E. coli	*Escherichia coli*
S. aureus	*Staphylococcus aureus*
HBV	hepatitis B virus
DNA	Deoxyribonucleic acid
mg	Milligrams

ml	Millilitre
Wt.	Weight
HCAI	Health care-associated infection
pH	Potential of hydrogen
MRSA	Methicillin-resistance *Staphylococcus aureus*
EPA	Environmental protection Agency
CDC	Centers for disease control and prevention
FDA	Food and Drugs administrative
RTU	Ready to use
RTUDW	Ready to use disinfectant wipes
ID	Infectious disease

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

In our growing life the risks of infection increase day by day rather than decline. The situation is getting worse indicated the growing number of infections. Public should be informed in a fair and responsible manner. Everyone should be aware of difference between "Dirt" and "Contamination". Study data show that disinfectants have become more important in household in the context of “hygienic options” to prevent infection disease [1].

Disinfectant is a common practice of removing pathogenic microorganisms using chemicals. The disinfectant process involves a chemical to remove almost all of the bacterial contaminants, but it is unable to remove endospores present in nonliving objects [2].

Healthcare acquired infections (HCAIs) happen due to transmission of pathogen found in hospital (nosocomial) from mostly touch surface in hospital and surgical equipment, these are the cause for substantial patient illness. In recent study it shows that nosocomial bacteria, including methicillin resistant *Staphylococcus aureus* (MRSA), *Clostridium burdile,* norovirus, vancomycin-resistant *Enterococcus*, and *Acinetobacter* etc, which patients disperse and make whole hospital contaminate [5]. An operative practice of dusting and disinfecting, such such as chemical disinfection plays a major role in prevention cross contamination to the spread of HCAIs. Among all disinfection methods, the use of a chemical disinfectant widely distributed in the food industry, hospitals and health facilities because of its simple use as wide antimicrobial activity [6]. In use of disinfectants in practice, “ready to-use” disinfecting wipes (RTUDW) are increasingly accepted to cleaning of areas that are less affected by their ease of use and reliable performance [6]. For surface disinfection RTUDW is approved to be use. The results in many research projects shows that it has a positive anti-bacterial effect in several cases [7].

Antibiotics are the synthetic organic substance which occur naturally, which prevent some bacteria at very low concentrations and antiseptics are the substance that prevent the growth of microorganisms in or on living tissue (e.g. those who work in hospitals, surgical instruments)

and disinfectants are also same but these are the substance that are used on nonliving objects or surfaces. Disinfectants may work as sporostatic but necessarily as sporicidal [8].

Antibiotics, sterilizers, sanitizers, antiseptics and germicides are chemicals used for biohazardous pollution. Sometimes these words look the same, but not always. The effectiveness of all disinfectant is based on several factors: 1) live load (amount of dirt and other surface contaminants), 2) microbial load, 3) type of creature, 4) type of areas to be disinfected (i.e., entry or inactivity), and 5) bacterial concentration, pH, temperature, contact time with nature moisture. On the basis of these factors the disinfectant is considered high, medium or low antibiotic. Disinfectants are indispensable part of hospitals and other facilities for diagnosis and other instruments, they participate in the process to avoid infection, and in nosocomial diseases. chemicals (or "biocides") found in these products, most of which has long been used for antisepsis, disinfection, and preservation [9].

Disinfectant as a key component of disinfection act have a significant impact on the process of eliminating decontaminants. The property of killing bacteria of disinfectants works in two separate ways: growth inhibition (e.g. bacteriostatic, fungistatic) and lethal action (sporicidal, bactericidal, fungicidal, and virucidal effects). Every disinfectant have some some positive impact as well as negative impact [4].

Regular cleaning is the indispensable act of controlling the growth of microbes and preventing it from making biofilm. The cleaning process removes the organic matter waste from the surface [10]. Cleaning can eradicate about 90% of the bacteria without killing them. That’s why, it is important that disinfection is done in the background cleaning, preventing binding of bacterial cells to local touch and reducing the amount of micro-organisms found on surface. There are different ways to kill germs in places: chemical, physical and mechanical [11].Most frequent used mthod is Chemical methods to control pathogen such as *Escherichia coli*, *Listeria monocytogenes*, *S. aureus* and *Salmonella*, as well as decay micro-organisms such as *P. aeruginosa* [9].

Variety of disinfectants are present : QACs, halogen extraction agents, peracetic acid, hydrogen peroxide (H2O2), organic extracts, etc. Disinfectant effectiveness tests include
three steps: i) testing with planktonic cells, ii) test with carrier cells and iii)

plant tests (in situ) [9].

Now a day many type of disinfectant is found among those air disinfectants are also very useful, usually chemical substances that can kill germs through microorganisms suspended in the air. Antbiotics are often thought to be limited to outdoor use, but this is not the case. Studies have shown that airborne microorganisms can be killed using dilute bleach fog. The disinfectant should be dispersed as an aerosol or vapor in a sufficient amount of air to cause the number of microbes found to be significantly reduced.

Alcohols, generally isopropanol or ethanol, are occasionally used as a decontaminant, but frequently as an antiseptic, the difference is that alcohol is more often used in living tissues than in non-living. These alcohols are not harmful but can be a fire hazard. In addition, they have limited residual activity due to evaporation, which leads to shorter communication times without the area being submerged. Besides,their activity is restricted in the existence of organic material [12].

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. LIST OF SOME COMMONLY FOUND DISINFECTANT:

Each chemical disinfectant has its own Hazards, Characteristics, and Efficiency against different microorganisms. And in the following paragraphs, some of the common chemical disinfectants and their characteristics are described.

2.1.1 Chlorhexidine - Biguanide disinfectant are known for the capability to destroy the microorganisms by damaging the permeability of cell membrane and Chlorhexidine is one of them. In the presences of organic matters Chlorhexidine can maintain it is effectiveness. Chlorhexidine is slightly less effective when it is considered for fungicidal, bactericidal and veridical and it is non-irritating.

2.1.2. Ammonium Hydroxide - Ammonium Hydroxide is usually found as household ammonia and has no colour property at all. It is caustic at high concentration and also has a lousy odour. Alkalis are used as disinfectant against numerous pathogens which also covers most bacteria and viruses and Ammonium hydroxide is an alkali. In low temperature disinfectant capacity of alkalis is slow but it increases with the rise of temperature. Alkalis cannot be used against the non-enveloped viruses and bacterial spores because they may ineffective against them.

2.1.3. Ethyl Alcohol - Ethyl Alcohol is a high flammable liquid and volatile in nature, it is colorless and also known as Ethanol in day to day language. Ethanol is an effective disinfectant and it is easy availability, lack of chemical residue and rapid killing action makes Ethanol ideal for disinfection of medical equipment. With the concentration range being 60% to 90% solution with water, the sudden drop in the activity of Ethyl Alcohol is seen when diluted to below 50% in concentration. Ethanol and solutions are flammable and can be easily ignited if it contains more than 50% ethanol. Danger of health hazards is present when human body over exposed with ethanol.

2.1.4. Benzalkonium Chloride & Cetylpyridium Chloride - Benzalkonium Chloride and Cetylpyridum Chloride are quatenary ammonium compounds that are used as disinfectant in large number. They are effective when used against fungi, bacteria and enveloped viruses. Benzalkonium Chloride and Cetylpyrdium Chloride are cationic detergents, and reported as non-toxic during used as diluted concentration. However, continues contact with disinfectant containing high concentration can lead to skin irritation. It has limited effect keeping surfaces bacteriostatic for a longer period of time. These are the common ingredients in disinfection of farms, home, offices, and most importantly the hospitals. The chemical property of them considered stable for storage but can be inactivated by hard water, anionic detergents, soaps, and organic matters.

2.1.5. Glutaraldehyde (1.5- pentanedial) - Glutaraldehyde disinfectant is very efficient during opposing of viruses, bacteria and fungi. Glutaraldehyde broadly used in cooling water system, food industry, medical field, paper pulp industry, cosmetic industry, poultry and microbiological field due to its antimicrobial property. This biocide is excessively used because it is non corrosive to metals, rubber and stainless steel etc. Organic solutions and solvents are stable for long period of time and Glutaraldehyde is soluble in water. It is impressibility is majorly depending on pH and temperature, when temperature is high and pH is greater than 7 it is performance reach to best levels. When it comes to efficiency in the company of organic matters soap and hard water then Glutaraldehyde is more effective then Formaldehyde. There is a potential danger of health problems which includes exacerbation of asthma, and skin sensitization can be seen when exposed with Glutaraldehyde liquid or vapor.

2.1.6. Calcium Oxide (CaO) - In general term Calcium Oxide is also known as lime or quicklime which is a white caustic solid. It dissolves in water and form calcium hydroxide and generates heat. It can generate sufficient heat to ignite nearby combustible substances when contacted with water or moisture. Calcium oxide vigorously reacts with halogens and acids. Calcium Oxide forms an average strong alkali when dissolved in water that has a biocidal effect on some virus and bacteria. This chemical is usually used as disinfectant for animal corpse. To disinfect living areas, slaked lime solution is used (i.e. Calcium Hydroxide). Respiratory Discomfort can be caused if Calcium Oxide dust is inhaled and it's acidic and can burn the skin and eyes if contacted.

2.1.7. Cresol & Hexachlorophene - They are Phenol disinfectants. They have minimal effect against viruses but excellent in wiping out of bacteria, they are inactive against bacterial spores. Area where organic materials are present in abundance then this disinfectant is applied. Phenol compounds are active elements in almost every household disinfectant and can be easily found in surface cleaner and scrub soaps. Phenol compounds has unique odor and may be harm the skin or eyes. Breathing, injecting and application on skin with high concentration of phenol can be lethal and doing the same may cause circulatory collapse, vomiting, convulsions, coma and paralysis.

2.1.8. Calcium Hypochlorite - Calcium Hypochlorite is largely applicable chlorine compound against algae, bacteria, fungi and other microorganisms because it's highly effective. When it comes to chlorinating swimming pools and to treat sewage and water supplies, Calcium Hypochlorite is the main element for that, it is also regularly uses as sanitizer in industrial applications and as a bleaching agent. Usually Calcium Hypochlorite consist 65% available chlorine and is a strong oxidant because of that when it contacts with reducing agents, acids or combustible materials, there is a heavy risk of explosion and fire is present. With other elements like ammonia, nitrogen compounds and amines etc. it reacts violently and causes explosion hazard.

2.1.9. Sodium Hypochlorite - Sodium Hypochlorite is active in removing bacteria, fungi and viruses and active element in commonly used bleach. It is one of the most used chlorine containing disinfectant. For cleaning of surface, purification of surface, water disinfection, odor removing and bleaching Sodium Hypochlorite is used on a utmost scale. Usually there is 5.25% solution of Sodium Hypochlorite is present in liquid chlorine bleach which is uses as disinfectant. Dissolved hypochlorite solution rapidly loose it's potential therefore it is necessary to prepare freshly just before use. Sodium Hypochlorite is acidic and corrosive to metal surfaces and high concentration.

2.1.10. Hydrogen Peroxide - Hydrogen Peroxide is a colorless liquid in normal conditions. It is one of the most common oxidizing and bleaching agents and largely uses in making deodorants, as a rocket fuel, water and sewage treatment and as a disinfectant. The preparation considered as bactericidal, fungicidal, and virucidal only when it contains 15 % to 20% of Hydrogen Peroxide, it is sporicidal at high concentration. In cleaning of scrapes and human cuts the diluted form if Hydrogen Peroxide is generally used. Concentrated Hydrogen Peroxide is highly explosive and reactive and may also cause chemical burn of eyes and skin.

2.1.11. Ethylene Oxide - In sterilization of heat sensitive instruments like medical equipment's and preparation the Ethylene Oxide is primarily applied. It is very useful and efficient against large variety of fungi, bacteria and viruses. Ethylene Oxide is highly toxic, colorless and odorless gas and possesses the property of being highly explosive and flammable. It has an explosion concentration range of 3% to 100% by volume in air. Ethylene Oxide is a carcinogen and may hurt the skin and eyes and may also cause of damage of respiratory and nervous systems.

2.1.12. Ortho-phthalaldehyde (OPA) - It is chemically related to Glutaraldehyde and is solid and light yellow in nature. High pH makes it more effective against microorganisms just like Glutaraldehyde and OPA is also used as an alternative of glutaraldehyde. It can provoke the pre-existing asthma and may cause sever irritation in skin and eyes. OPA is stable at a wide pH range and inflammable in nature.

2.1.13. Iodine - Iodine compounds can terminate a variety of fungi, bacteria and virus. Iodine are generally formulated with soaps because it is relatively safe. Concentrated Iodine compounds can damage rubber and metal, may irritate the skin and stain clothes. For scrapes and skin cuts, tincture of Iodine is applied. With the help of organic debris and quaternary ammonium compounds, Iodine agents can be inactivated.

2.1.14. Formaldehyde - Formaldehyde is a carcinogen. It is a colorless toxic gas with pungent and suffocating odour at room temperature. This chemical is freely water soluble. Commercial Formaldehyde chemical is produced and sold as a formalin (aqueous solution) which consist of 37% to 50% of Formaldehyde by weight. Aqueous Formaldehyde is uses as a preservatives or disinfectant. Formaldehyde can be used as a agent to disinfect surface, object and air beside being liquid disinfectant.

2.1.15. Iodophor - It is preparation of elemental iodine complex with polymer Carrie which is complexing agent of high molecular weight. Resulting complex offers a sustained release of iodine in aqueous solution. Its bactericidal efficiency is slow and are hard to inactivate by organic matter then element iodine and commonly used as a disinfectant. Povidone-iodine (PVI) is a easily feasible Iodophor, generally prepared as 7.5% to 10% solution. Dilution increases iodine concentration therefore formulation with lower concentration have good antimicrobial activity, with the increase in free iodine, the grade of frustration of skin is also increases.

2.1.16. Per acetic Acid (Peroxyacetic Acid) - it is colorless liquid with sour vinegar like odour. It does not possess any environmental harm because Per acetic acid decomposes to water, oxygen and acetic acid. It is a powerful oxidizing agent which kills numerous microorganisms in no time, concentration of only 0.2% of Per acetic acid is enough and effective against microbes which also includes bacterial spores, it is also effective at low temperature in the occurrence of organic matter. Peracetic acid may cause of material discoloration and metal corrosion, but it is uses as spray or as a mop on solution.

2.1.17. Isopropyl Alcohol - Isopropyl Alcohol is commonly known as Isopropanol, it is colorless liquid with identical odour like acetone or ethanol and Isopropyl Alcohol is also highly flammable. It can be found in rubbing alcohol, cleaning agents and alcohol sponges. 70% of Isopropyl Alcohol present in rubbing alcohol. It is may cause irritation on eyes and mucous membranes and also causes of eczema when long contact made with this chemical.

2.1.18. Paraformaldehyde - Paraformaldehyde is a white powder with odor property of formaldehyde. To decontaminate laboratory facilities and to disinfect sickrooms, clothes, sickroom utensils and linen Paraformaldehyde is used for more than 30 years. Paraformaldehyde releases formaldehyde gas when heated which is an excellent disinfectant. It can be irritating the skin respiratory system and eyes, exposure to higher concentration of Paraformaldehyde may cause pulmonary edema.

2.2 MODE OF ACTION

2.2.1Alcohol

The most suitable clarification of killing microbes by alcohol is proteins denaturation. This process can be maintained by the consideration that ethyl alcohol which is a drying agent is found to be less effective than the combination of water and alcohol as in the presence of water proteins are denatured very easily. The denaturation of protein is also compatible with the observations that the dehydrogenases of *Escherichia coli* is damaged by the alcohol, and the lag phase of *Enterobacter aerogenes* is increased by ethyl alcohol, and by adding some amino acids the lag phase can be reversed. The Bacteriostatic action was believed to promote hindrance for the production of metabolites which are necessary or rapid cell division [13].

2.2.2 Chlorine

Electronegative property of chlorine oxidizes peptide links and denatures proteins. Hypochloric acid is produce by Hypochlorite and chloramine present in water, which involve in decomposition. Chlorine and oxygen both are involved and thiol groups are oxidized[14]. The exact process by which microorganisms are destroyed by chlorine has not been explained. There are a number of factors which can result in the inactivation of chlorine: amino acids ring chlorination, oxidation of sulfhydryl enzymes and amino acids, damage of intracellular substances, decreased nutrient uptake, decreased oxygen uptake, hindrance of protein synthesis, oxidation of respiratory constituents, decrease in adenosine triphosphate production takes place, breakage in DNA, and low DNA synthesis. The exact microbicide process of chlorine might contain a combination of these factors or the consequence of chlorine on dangerous site [11].

2.2.3 Aldehyde compounds

Formaldehyde

Formaldehyde perform on nucleic acid by Alkylation and on protein by denaturation: Except for guaniribo-deoxyribonucleotides, formaldehyde is identical in terms of action at the ribo- and deoxyribonucleotide levels. The 5' dGMP (deoxy-guanosine monophosphate) interacts quickly with formaldehyde than 5' GMP. The equilibrium shifts towards hydroxymethylation and the reaction with receptive nucleotides occurs rapidly. This action is reliant on pH, It works better in terms of alkaline pH and less fine in terms of neutral or acid Ph. [14].

Microorganisms are inactivated by formaldehyde when the amino is alkylated and sulfhydral groups of proteins and ring nitrogen atoms of purine base [11].

2.2.4 Hydrogen Peroxide

The critical hydroxyl free radicals are produced by oxide due to which attack on membrane lipids, DNA, and other essential cell components takes place. The cytochrome systems possessed by the aerobic organisms and facultative anaerobes produce catalase, which protects the cells from metabolically oxide by the degradation of oxide to oxygen and water. This resistance is overpowered by concentrations utilized for disinfection [11].

2.2.5 Phenol

The phenol acts especially on the semipermeable membrane and therefore the intracytoplasmic enzymes are inactivated by formation of unstable complexes. The membrane phospholipids trap the lipophilic molecules. the subsequent mechanisms are included: the cell constituents (nucleic acids, glutamic acid) are sat free within the foreign media if concentration is low. The disinfectants inhibit permeases, thus causing denaturation of the bacterial proteins and lysis of the semipermeable membrane, if the media concentration is high. within the instance of ***Bacillus megaterium,*** the intracellular solutes are liberated from the testing cell or during growth like protein derivatives and tiny solutes acts as a secondary consequence of interlinkage of enzymes certain to cytoplasm membrane [12].

2.2.6 Quaternary ammonium compounds

Quaternary ammonium compounds (QUACs) impairing permeability by binding with phospholipids and proteins membrane. The ability of the bacterial cell to absorb molecules which stimuluses sensitivity in the following ways:

- The killing effects of bacteria of quaternary ammonium with an alkyl chain is related to lipophilicity and ranges between C12 and C16 for Gram positive and negative bacterial strains.
- Lipoproteins and liposaccharides are present on the outermost layer of peptidoglycan on *Pseudomonas spp*. and *Bacillus spp.* While many active compounds have less inhibitory effect on it.
- The maximum presence of neutral lipids and phospholipids in Pseudomonas species rises the resistance. Benzalkonium chloride content is responsible for more permeability of cell. The same sight is reported in *Enterobacter cloacae*.
- The final result of Bacteria which are gram positive becomes restricted to the wall proteins due to which it is able to cross and damage the membrane.
- In Gram-positive and Gram-negative bacteria uniform absorption has seen, compared to an excess in permeability and decreased viability (e.g. cetyltrimethylammonium in *E. coli*). Electron microscopy exposes damage to Pseudomonas aeruginosa at outer membrane level.
- Cetyltrimethylammonium causes leakage to metabolites by lowering cell mass (physical damage and energy conversion) in *Staphylococcus aureus* [12].

2.3 BASIC PRINCIPLES OF DISINFECTANT

Programs to make disinfection in addition to the required information, effective disinfection procedures, guidelines or regulations require a clear, concise plan of action for each disinfectant application. The plan should explain the program's objectives and specific microorganisms that must be eliminated. It should describe the pre-disinfection cleaning procedure, safety measures, mixing and application instructions, and disinfection procedures after which the process will be measured. The application should also provide details on the documentation required to provide regulatory certification.

Regulatory virus control and control policies. Regulatory monitoring may be required to ensure the following:

- High efficiency in product usage

- Use of all safety measures for humans, animals, equipment and the environment - pre-disinfection cleaning

- consciously apply antibiotics in the right places.

At the policy level, disinfection procedures and regulations should be reviewed and evaluated regularly with a view to rapidly evolving technologies and changing public standards regarding human safety, residual hazards and environmental awareness [15].

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2.4 TOXIC EFFECTS OF DSINFECTANT

There is some imitation of contraceptive methods s a result, the disinfection method became the method of a comprehensive and widely utilized method in hospitals, indoor space (kitchens and toilets), and outside house to eradicate infectious organisms. These disinfectants are a widely available, inexpensive range, and use in many places and objects without the need for an machine tools. A huge number of chemical disinfectant used in health care and home making that add chlorine quaternary ammonium compounds (Quats) alcohol, chlorine compounds (bleach), peracetic acid, aldehyde, improved hydrogen peroxide, phenol compound and Iodophor. Commercially available disinfectants have active compounds based on these chemical are unique and appropriate and get listed with the US Environmental Protection Agency (EPA) or can disregarded by the Food and Drug Administration (FDA) [16].

TABLE 1. Product Listed by EPA [2].

Active compounds	**Effective contact time in seconds**	**Formulation**
Ammonium carbonate	360	Quickly utilize
Citric acid	60	Quickly utilize
Isopropanol	30	Rub
Peroxyacetic acid	60	Can be diluted
Hypochlorous acid	600	Quickly utilize
Hydrogen peroxide	300	Can be diluted
Hydrogen peroxide	600	Can be diluted
Ethanol	120	Quickly utilize
Octanoic acid	120	Can be diluted
L-lactic acid	600	Quickly utilize
Triethylene glycol 5	300	Pressurized fluid
Sodium hypochlorite	600	Can be diluted
Glycolic acid	600	Saturated material
Phenolic	600	Can be diluted
Quaternary ammonium	600	Can be diluted

(Quats)		

As we all know due to Covid we are using enormous amount of disinfectant in our daily lifestyle which might lead to next disaster in public health and ecosystem. Earlier so many research has been done with most widely used disinfectant compounds, that is, quaternary ammonium compound, hydrogen peroxide, sodium hypochlorite, alcohol, and aldehyde. All of them are associated with the bigger risk of chronic obstructive pulmonary disease, eye irritation and asthma on health workers and person when used continuously [17].

Remains of chemical that left on the surface can be inhaled usually they play role in poor in-house air quality with harmful effect for asthma, allergy infected , or sensitive people [18]. These chemical remains can be carcinogenic and cancer, respiratory disorders (including occupational asthma), reproductive diseases, irritation in eye and skin, central nervous system (CNS) damage, oxidative harm, and other public health effects. Quaternary ammonium compounds possibly cause infertility in both male as well as female reproductive system [19]. Some scientists claims that 5% cancer and 30% asthma in children due to more chemical contacts [20].

Due to current situations(Covid19) and because of nervousness person use many disinfectants at same time to decontaminate their houses and belongings. There are surveys which recognized significant information about safe training of how to clean and disinfect their houses. Serious harmful properties can effect because of combination of different disinfectants; they may create lethal gases. Direct contact to these gases can encourage respiratory disorder(asthma) and prolonged bronchitis. The combination of ammonia with bleach -based disinfectants produces the chloramines and might be ammonia that can vaporize. Instead, when an acid-based cleaner was combined with bleach, it can generate Hypochlorous acid or chlorine gas, which causes acute lung injury even inhaling in small amount. In addition, chlorine disinfectants mixed with nitrogen forms monochloramine or dimethylnitrosamine, which have can cause cancer [21]. Combination of ethanol and sodium hypochlorite produces Methylene chloride (chloroform), they are hazardous and harmful when inhaled and for skin when come in contact. To reduce these challenges, suitable guideline should be presented in the human ownership about the

dangerous effect of disinfectants, how to combine, when should wear PPE(Personal protective equipment) kit, confirming suitable airing, and storage and away from children and pets [22].Components which are being utilized in commonly available disinfectants are powerfully stimulated by EPA to be “biodegradable”. For cleaning products many types of profit are there if there are chemicals which are biodegradable, however many types ideas are also combined with it.

After use from the surface when “Biodegradable” disinfectants are not washed properly, leaves an active chemical residue. Due to this chemical residue the surface acts like a sticky and attracts soil, sand, oil, dermis and pollens which act as a source of food for molds and other microorganisms. That’s why, a well exquisite wiping and decontamination technique always consists of 2 steps; cleaning followed by application of a disinfectant/sanitizer.

Let’s take an example of Benzalkonium disinfectants. Disinfectant which have Benzalkonium is based always on its structure of ion which are positively charged. Quat can become electrically neutralised and its bacteria killing action may entirely inoperative, if there is any amount of anionic detergent remains present on a surface before the use of Quaternary ammonium compound, then the complicated detergent ‘quat remain’ may serve as food source for development of microorganisms. These microbes may colonize when allowed to grow and making colonies called biofilms [23]. These biofilms have a habit to flourish in atmosphere where humidity, dust and dirt are in continuous interaction on a surface. As we all know COVID-19 gets increase by air borne route. The practise of scattering disinfectant and alcohol in air, on surroundings, on roadsides has not positive impact. Instead these harmful compounds can enter the sewers and can contaminate our resources of drinking water. If the level of chlorine disinfectant gets increased, it might openly affect organisms by damaging their cell wall and by oxidation can destroy protein. Also the compounds present in disinfectants may bind with other material to formulate hazardous secondary by products as THMs (trihalomethanes) or Dibromoacetic acid. Specially to aquatic organisms these by products are proved to be very toxic. China uses ozone in hospitals or homes against SARS-COV-2. Several serious health issues can be caused due to maximum contact of trioxide (ozone) and some air pollutants. Persistent, bio accumulative and toxic chemicals(PBTs) are some unsafe chemicals contained by

some of the cleaning products which are termed as a dangerous waste and /or contributes to ecological contamination while their manufacturing, use or discarding [4].

2.4.1 SOME CHEMICAL DISINFECTANTS AND THEIR TOXICITY VALUE ON HUMAN

TABLE.10 Toxicity of different chemical on human

COMPOUND NAME	TOXICITY ON HUMAN	REFERENCES
Ammonium hydroxide	50ppm	[24]
Benzalkonium chloride	10%	[25]
Calcium Hypochlorite	<150gm	[26]
Calcium oxide	$5mg/m^3$	[27]
Chlorhexidine	2%	[28]
Cresol	8gm	[29]
Ethylene oxide	400ppm	[30]
Hydrogen per oxide	$10mg/m^3$	[31]
Sodium Hypochlorite	300ml(>10%)	[32]
Iodine	Intake >1.1mg/day	[33]
Peracetic acid	5ppm	[34]

2.5 CHEMICAL SAFETY

To ensure the safety and health of workers involved in disinfection, a carefully planned chemical safety plan is essential.

The purpose of the chemical

A security system to systematically identify and investigate potential risks to reduce the risk of adverse health and safety consequences due to exposure to chemicals in the workplace. In the system, the chemical hazards of disinfectants must be identified first. The risks associated with these risks are assessed to look at working conditions with the employees involved. It is worth it Preventive and / or control measures are set to eliminate or reduce the methods of risks, with their functionality being regularly assessed and reviewed. The details of the risk associated with safety measures should be informed to all affected employees. The chemical safety plan should also include other things like emergency response planning and staff training.

A chemical safety plan should be planned and integrated into everything Workplace safety management system in order to operate effectively implementation. Employers must provide adequate capacity and resources in the construction, design and maintenance of the system [13].

2.6 MECHANISMS OF RESISTANCE AGAINST DISINFECTANTS

Every microorganism has unique ways to response to disinfectants. This is not the case at all it is amazing to look at the structure of different cells and physiology. Traditionally, the tendency of bacteria to disinfectants have been verified on the basis of the recent work (Fig. 1). Because different species respond to it in contrast, it is easier to look for bacteria, fungi, germs and protozoa separately.[16]

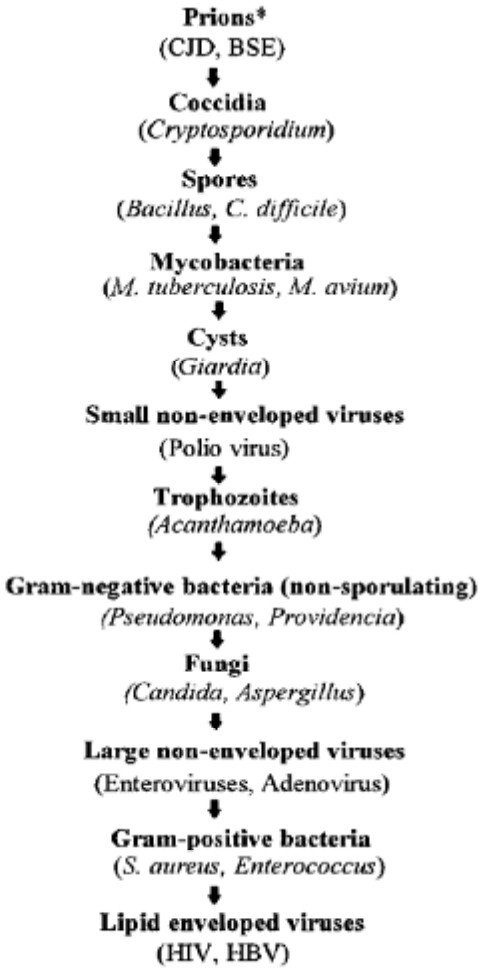

FIGURE 1 Order of resistance to Disinfectants in descending way [14].

2.6.1 How Bacteria Resist against Disinfectants

In past days, great strides have crossed to fully understand the reaction variable bacteria (mycobacteria, undiagnosed bacteria, and bacterial spores) to disinfectants [35].

By which the result concluded that resistance may be a natural asset for body (internal) or acquired by conversion or gaining of plasmids (replication, additional DNA) or transposons (chromosomal or plasmid fusion, it is infused DNA cassettes). Internal opposition is grammatically correct bacteria, bacterial strains, mycobacteria, and, less certain conditions, staphylococci **(Table2).** Found that plasmid resistance is strongly associated with mercury.[36]

TABLE.3 Resistance mechanisms in bacteria to disinfectants by intrinsic way [14].

Resistance types	Examples	Resistance mechanism
Impermeable Gram-negative bacteria	QACs, triclosan, diamines	Bacterial cell comprises of an outer membrane that provides the bacteria with the resisting power against antiseptics by inhibiting the cells to uptake disinfectants.
Mycobacteria	Chlorhexidine, QACs Glutaraldehyde	The cell wall of Mycobacteria is waxy that inhibits the entry of biocides.
Spores of bacteria	Chlorhexidine, QACs, phenolics	Bacterial spores are resistant to the action of disinfectants due to the presence of spore coat(s) and cortex.
Gram-positive bacteria	Chlorhexidine	The presence of mucoexopolysaccharide in gram positive bacteria may decline the diffusion of disinfectant in the cell there by providing them with the resisting ability against antiseptics.
Inactivation (facilitated chromosomally)	Chlorohexidine	Chlorhexidine breakdown may be the responsible for resistance.

1.6.1.1 Resistance Mechanisms of Bacteria by Intrinsic way

The outer layers of the cell should fall off for disinfectant molecule to reach its goal site. Exact situation the constituents of these layers depends on type of body as well can serve as an entry barrier, in which case reduced discovery [37]. Alternatively but rarely, combined enzymes can cause degradation of compound [38]. Therefor Intrinsic (innate) resistance is a natural, controlled chromosomally substance of the bacteria that helps to skip disinfectant. Gram-negative bacteria are often more common resistance to gram-positive substances, such as staphylococci**.**

MIC value of Benzalkonium chloride against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 0.5,50,250mg/ml respectively. Chlorhexidine against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 0.5-1,1,5-60mg/ml respectively. Hexachlorophene against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 0.5,12.5,250mg/ml respectively.Phenol against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 2000,2000,2000 mg/ml respectively. Propamine isethionate against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* are 2,64,256mg/ml respectively [39].

1.6.2 Resistance Mechanisms of Fungal to Disinfectants

Compared with bacteria, fungi have little known ways by which it can prevent the action of disinfectants. There are two common ways of resisting. **(Table 3)**: (i)internal, natural resistance property or physical development [25]. (ii) Acquired discovery, With one method of internal resistance, the cell wall introduces a block to decrease or eliminate an intrusion of an antibacterial agent. The evidence so far has faded, but available data link cell walls glycan, wall width, and increased permeability in the *Saccharomyces cerevisiae* to chlorhexidine **(Table 4) [26]**.

TABLE.4 Fungal mechanisms of resistance against disinfectants [40]**.**

Resistance types	Possible way of mechanism	Example
Intrinsic	Exclusion	Chlorhexidine
	Inactivation of enzyme	Formaldehyde
	Modulation of Phenotype	Ethanol
	Efflux	Not demonstrated to date
Acquired	Some Mutation	Some preservative
	Inducible efflux	Some preservatives
	Mediated Plasmid responses	Not demonstrated to date

Protoplasts of this creature arranged by glucuronidase with the occurrence of b-mercaptoethanol are maintained by the concentration of chlorhexidine less effective than "normal" (complete) cells. In addition, the age of the culture stimuluses the response of *S. Cerevisiae* to chlorhexidine; the cell walls are not much complex in the vertical phase than in the logarithmic growth phase, taking at least [$_{14}$C] chlorhexidine gluconate [14].

TABLE.5 *S. cerevisiae* Limitations affecting the response to chlorhexidine*a.*[14]

Parameter	**Role in susceptibility of cells to chlorhexidine**
Cell wall composition	
Mannan	No role found
Glucan	At low concentration Possible significance get active against whole cells, lyses of protoplast occur by chlorhexidine
Cell wall thickness.	Cell increases in older culture, reduced chlorhexidine uptake responsible for decreased activity
Relative porosity	Decreases in cells of older cultures: reduced chlorhexidine uptake responsible for decreased

	activity
Plasma membrane	Changes altering CHG susceptibility, not investigated to date

Adhesion of the yeast wound wall is pretentious by its composition of chemical, and the wall acts as a blockade or modulator in transporting of numerous agents. DeNobel et al. [28] use of fluorescein isothiocyanurate (FITC) dextrans and periplasmic enzyme invertase as pointers of cell wall of yeast porocity. Intact *Saccharomyces cerevisiae* cells can endocytose FITC dextrans of 70 but not of 150.

These findings **(Table 5)** may provide an overview of cellular features that alter *S. cerevisiae* to chlorhexidine. Mannan mutants of *Saccharomyces. cerevisiae* shows the same level of sensitivity to chlorhexidine as a parental. The glucan layer is protected from b-glucuronidase by mannan proteins, but this effect is overcome by b-mercaptoethanol. Mannan protein contains two components, sodium dodecyl mannan proteins containing soluble sulfur and sodium dodecyl sulfate- insoluble, insoluble glucanase: the last wall determined by the cell porosity. Therefore, glucan (and possibly mannan proteins) shows an important part in deceiving recruitment and later employment of chlorhexidine in *Saccharomyces. cerevisiae*., *Candida albicans* are less complex and takes less chlorhexidine by [$_{14}$C] in general, but only a few studies with this body and fungus have been done. Yeast that grows under different conditions has varying degrees' ethanol sensitivity. Cells are linoleic acid-rich plasma membranes are more unaffected to ethanol than they are oleic acid-rich cells, from which they are derived it have been proven that a water-repellent sheath adds ethanol resistance. The mold is usually highly resistant to yeast and is more unaffected than nonsporulating bacteria**.** Molds spores, although more resistant than non-nonsporulating bacteria, are less unaffected than bacterial spores to disinfectants. Which are appealing to attempts to predict the formation of the cell wall provides a great level of internal resistance to these microorganisms [14].

The lethal concentration of Benzalkonium chloride against *Candida albicans, Penicillium chrysogenum, Aspergillus niger* are 10,100-200,100-250mg/ml respectively. Cetrimide/CTAB (cetyltrimethylammonium bromide) against *Candida albicans, Penicillium chrysogenum,*

Aspergillus niger are 25,100,250mg/ml respectively and Chlorhexidine against *Candida albicans, Penicillium chrysogenum, Aspergillus niger* are 20-40,400,200 respectively.[39]

1.6.3 Mechanisms of Viral Resistance to Disinfectants

Grossgebauer first reviewed studies of the outcomes from disinfectants on viruses. They are possibly the target of the virus viral envelope, which carries the usual membrane unit and lipids, capsid, essentially a protein naturally, and inheritance. An important plan was put in action in 1963 [29] where it was proposed that the weakness of the virus against disinfectants can be based on viruses that are "lipophilic" nature, because lipid envelope are present (example - herpes simplex virus) or "hydrophilic" lipid envelop is absent (e.g. - polio virus). From lipophilic type disinfectant, lipid covered viruses are sensitive, such as 2-phenylphenol, chlorhexidine, cationic surfactants (QACs) as well as chloroform and ether. Virus are divided into three strains groups by Klein and Deforest [30] (Table 8), i (Containing Lipid), ii (Nonlipid Picornaviruses), iii (Some Non- Enveloped Viruses that are larger than those in group ii) and disinfectant in two categories, broad spectrum those that do not work all viruses and lipophilic failures activating picornavirus and parvovirus [14]

Capsid proteins are primarily proteins by nature, and biocides such as aldehyde, hypochlorite, ethylene oxide, and H_2O_2, which reacts strongly with amino acids or Sulfhydryl groups may active against both enveloped and non-enveloped. However, the damage capsid of virus may become the result of emission of tentatively infectious nucleic acid and that virus inactivity can lone be eliminated if nuleic acid is of virus and it is devastated.

Inappropriately, the influx of disinfectants on the other types of viruses and their synergy with viral constituents has been planned for a period of time, although, some particulars provided for research by inquiries with bacteriophages. To test the virucidal activity of disinfectant, Bacteriophages are suggested as an "indicator species" so, can play crucial part in this context; for example, repetitive expression of *E. coli* phage f2 to chlorine is said to surge it's resistance to disinfectant.

Thurman and Gerber [31] have debated changes on the activities of disinfectant on various viruses were described, and referred to building the reliability of the virus was transformed by the responding agent, viral capsule increase viral proliferation. Therefore, an effective way of

contraception is a "two-stage" disinfection program, while overwhelming the power of regeneration to brief the beginning decrease and then growth in disinfectant treated titer bacteriophage.

TABLE.6 Classification of Virus and effect against disinfectants[32].

Group of Virus	Lipid envelope	Examples of viruses	Effects of disinfectants	
			Lipophilic	Broadspectrum
I	present	influenza virus HSV, HIV, virus, rabies virus, Newcastle disease	Sensitive	Sensitive
Ii	absent	Non-lipid picornaviruses (poliovirus, Coxsackie virus, echovirus)	Resistant	Sensitive
Iii	absent	Other larger nonlipid viruses (adenovirus, reovirus)	Resistant	Sensitive

2.7 Minimum inhibitory concentration (MIC) DETERMINATION

This experiment involves determining action of a chemical agent, according to the sensitivity against microorganisms. The purpose MIC was developed for all chemical agents, using the old method of consecutive dilution. [41].

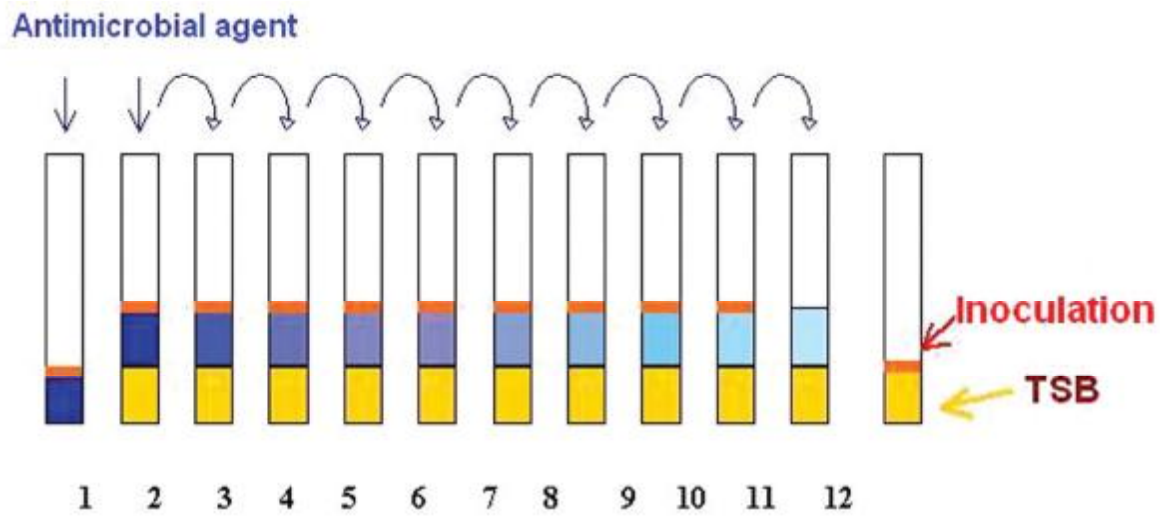

FIGURE.2 –Successive dilutions method for MIC [41]

The MIC value of Ethanol against *Bacillus stearothermophilus, Bacillus subtilis, E. coli, S aureus* are 87500mg/L (8.75%), 87500mg/L (8.75%), 65650mg/L, 87500mg/L (8.75%) respectively. And the combination of some chemical decreases the effectivity of Ethanol like Ethanol with Glycerin against *Bacillus stearothermophilus, Bacillus subtilis, E. coli, S aureus* are null, null, 87500mg/L (8.75%), 87500mg/L (8.75%). Ethanol with 1% of Iodine against *Bacillus stearothermophilus, Bacillus subtilis, E. coli, S aureus* are 87500mg/L (8.75%),87500mg/L (8.75%), 43750mg/L (4.38%), 43750mg/L (4.38%) respectively, but the combination of ethanol with 10% Iodine has the MIC values against *Bacillus stearothermophilus, Bacillus subtilis, E. coli, S aureus* are null, 43750mg/l (4.38%), null, 21870 (2.19%) respectively.[33]

When microorganisms exposed with same disinfectant their minimum inhibitory concentration differentiate. The medium culture (TSB) in experiment represents the presence of organisms, to work with microorganisms, increasing of difficulty for chemical agent is recommended. To know the culture moderate effect on every disinfectant which is used in unity, MIC limit trying to be resolute is a challenge. MIC determine a series of antimicrobial activity on given group of specific microorganism, recommending which group to be act as Biological indicator (BI). this

technique is also applied to pick commercial agent of chemical that delivers the best results when compared to rest of them.To test the effectiveness to all antimicrobials is important read decimation duration (value D).

Liquid disinfectant are accepted globally because of their price benefit. The chosen agent should be easily exploited nontoxic in the hospital areas, it is also used in areas like critical units and on daycare centers to keep themselves away from contaminants. Incorrect use of disinfectant mixtures can uplift resistance to pathogenic microorganisms, and to perform more batter programs for cleaning it is required to strengthen that. Interest and disinfection can cause infection, if not performed in right way.Here the values of MIC are indicating that how little amount of any chemical can easily kill the microorganism so, it can be harmful for human as well [33].

CHAPTER 3

OBJECTIVE

3.OBJECTIVE

- To check the efficiency of different Chemicals against Microorganisms.
- To achieve the different combination of chemicals to increase their antimicrobial property.
- To make the available disinfectant more efficient in less concentration.
- To gain the complete knowledge about handing different chemicals.
- To know the hazardous effect of rapid use of disinfectants.
- To make other people aware about the guidelines of using Disinfectants.
- To differentiate every type of chemical on basis of their use in different places.
- To select the best types of chemical for our houses.

CHAPTER

MATERIAL AND

METHODS

CHAPTER 4

STRETAGIES FOR METHOD

For each chemical/ combination of chemical 3 different agar plates are require. Plates should be keep in 3 different places to grow bacteria.

- First plate in Sterile condition.
- Second in closed room.
- Third in open area.

After 24 Hour bacteria growth need to count by CFU and then interest of chemical should be add to see. After 24hour further growth should be count and difference in growth rate should be calculate.

CHAPTER

CONCLUSION

5.CONCLUSION

This study is done to know more about the chemical we are using in our daily life as Disinfectants. On an average a human being come in contact with any type of chemical disinfectant very commonly. Usually people don't even know about their consequences and get in contact with it which may harm their body. Without complete knowledge we should not use any kind of chemicals as disinfectant. This study showed the hazardous effect of combination of different chemicals. Chemicals are harmful for microorganisms as well as human being, it is necessary to use every chemical with adequate knowledge. The combination of some chemicals like bleach with ammonia, bleach with acid-based cleaner, chlorine disinfectants mixed with nitrogen chloramine, bleach and alcohol are very hazardous and causes different types of respiratory, reproductive, cancer and many kinds of diseases.

We were trying to make the best combination of chemical with highest efficiency with less concentration to achieve good quality disinfectant for nature and for mankind.

CHAPTER 6

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6.REFERENCES

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CHAPTER 6

APPENDICES

6. APPENDICES

6.1 MATERIALS REQUIRED

Test tubes, Flasks, Chemical (based on our choices), Agar, Petri plate, Incubator, Available culture of commonly found microorganisms, Autoclave, Laminar air flow etc.

6.2 METHODS

6.2.1 MIC

MIC is minimum inhibitory concentration. It is done to determine the unusual resistance. It is done to check the susceptibility of given drug or sample.

It shows the lowest concentration of given sample which is require to kill bacteria. It is very important to check the antimicrobial property of sample.

Procedure:

1. Prepare 2 fold serial dilutions of the test compound up to the numbers you require and 1 Control which can be any known antibiotic in a micro dilution plate.
2. Create inoculum from the culture you brought with sterile swab, prepare Mcfarland standard and dilute the standard in given media.
3. Dispense the inoculum into microdilution plate with the serial diluted test compound and incubate the micro dilution plate.
4. Read the micro dilution plate to determine the MIC value.
5. Plate a portion of each well on appropriate agar media, incubate in optimum temperature and condition, and check for colonies.

6.2.2 Anti-bacterial Activity Test

- **Agar Well Diffusion Method**

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a well with diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100µl) of the sample at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

- **Growth And Maintenance Of Microorganisms**

The strain of given bacteria has taken from culture . The strain from the plate should inoculate in the nutrient broth and then the inoculum was left for 1 – 2 days at 30°C in the incubator. After the growth of bacteria in the broth, it is used to perform the agar well diffusion method with the given plant samples.

- **Aim**

Anti microbial test is performed in this case to see the case to see the inhibitory effect of the given sample.

Bacteria Used – Available bacteria.

Procedure

Preparation of Plates

- Nutrient agar media was prepared for 1 plate of 15 ml.
- It was then subjected it to autoclave.
- Immediately after autoclaving it was allowed to cool at 45°C to 50°C.
- The freshly prepared media was cooled and poured into plastic Petri plates.
- The agar medium was allowed to cool at room temperature.

Spreading of Bacteria

- The glass spreader and plates were put in a UV light.

- 10µl of the sample should be drop on the plates and spread with the spreader evenly on the surface of the plate so the bacteria are spread in each corner of the plate.
- It was dried for 4 to 5 minutes.

  Then the wells should have punched and 100µl of sample should be added to each of the wells with one of the well contained 10µl of antibiotics.