Antimicrobial activity and bio-potency of homoeopathy medicines against enteric pathogens

A Dissertation Submitted to the Department of Microbiology, Faculty of Biological Sciences, University of Dhaka, Dhaka 1000 Bangladesh, for the partial fulfillment of the requirements for the Degree of Master of Philosophy (M.Phil) in Microbiology

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CERTIFICATE

This is to certify that the dissertation entitled "Antibacterial activity and bio-potency of homoeopathy medicines against enteric pathogens" Submitted to the University of Dhaka in fulfillment of the requirements for the Degree of Master of Philosophy is a record of bonafide research work carried out by Mr. Md. Abdur Rahim under my supervision. The work is original due to the best of my knowledge and I believe that no part of the work has been submitted before for any other Degree or diploma.

Supervisor:

Dr. Md. Mahfuzul Hoque

DECLARATION

I do hereby declare that the dissertation submitted to the University of Dhaka for the Degree of Master of Philosophy is based on my own investigation, carried out under the supervision of Dr. Md. Mahfuzul Hoque, Professor of the Department of Microbiology, Faculty of Biological Sciences, University of Dhaka, and Prof. Dr. Suvamoy Datta, Head of the Department of Microbiology, Primeasia University and that this or any part of this work has not been submitted for any other Degree anywhere.

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Dedication:

I would like to dedicate my dissertation to
‘Dr. Samuel Hahnemann’
Father of Homoeopathy Science

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Md. Abdur Rahim

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List of Abbreviations

%	Percent
^{o}C	Degree centigrade
µg	Microgram
µl	Microlitre
mm	Millimeter
e.g.	For example
et. al	and others
g	Gram
L	Litre
ml	Milliliter
mg	Milligram
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
Merc cor-6	Mercurius corrosivus-6
Merc-sol-6	Mercurius solubilis-6
Heper Sulfur-6	Heparsulfuris calcareum-6
Sp.	Species singular
Spp.	Species plural
TSB	Tryptic soy broth
TSA	Trypticase soy agar
PBS	Phosphate buffer Saline
ZOI	Zone of Inhibition

Abstract

Homoeopathy medicines are used in many countries for the treatment of various human ailments. Homoeopathy medicines have no direct effect on some acute infectious diseases such as post operation infection, because it needs immediate recovery, but homoeopathy medicines may cure patients slowly forever. Homoeopathy medicines are very effective in case of chronic ailments. In the case where pathogens of infectious diseases become multidrug resistant, traditional antibiotics are failed to kill bacteria; in that case homoeopathy medicines may be an effective alternative, producing no toxic side effects & bringing about rapid recovery. Homoeopathy medicine actually gained its greatest popularity for its impressive successes in the treatment of infectious diseases in the19th century. It represents a different approach for understanding disease and health, for its potential of strengthening a person's immune system that fights against infection. Homoeopathy medicines have been using worldwide, because of trust on homoeopathy medicines. Some works on antimicrobial activity of homoeopathy medicines had been done worldwide, but a few reports or no reports are available so fur in Bangladesh especially on enteric human pathogens. The study was carried out *in vitro* for antimicrobial activity of homoeopathy medicines against human enteric pathogens. Five homoeopathy medicines were used such as Mercurius Corrosivus-6 (Merc Cor-6), Mercurius Solubilis-6 (Merc Sol-6), Pyrogenium-6, Hepar Sulfuris calcareum-6 (Hepar Sulf-6), and Sulfur-6. These all medicines were applied against *E. coli* O157:H7, *Salmonella typhi*, *Shigella dysentriae, Vibrio cholera. Staphylococcus aureus, Bacillus cereus and Micrococcus luteus* had been treated with the five homoeopathy medicines for finding positive reference organism. Based on sensitivity to all five homoeopathy medicines *Micrococcus luteus* was selected as reference organism for further study. *In vitro* antibacterial activity of five homoeopathy medicines were done by Kirby Bauer method, of which, Merc-Sol-6 and Sulfur-6 medicines showed zone of inhibition against human pathogens studied. Merc-Sol-6 showed zones of inhibition against *E. coli* O157:H7 (13.1±0.20/50 µl/disc and 20.09±0.21/100 µl/disc), *Shigella dysenterae* (10.5±0.33/50 µl/disc and 15.45±0.15/100 µl/disc), *Salmonella typhi (*15.3±0.18/50 µl/disc and 19.13±0.30/100 µl/disc),*Vibrio cholera* (14.33±0.24/50 µl/disc and 19.46±0.20/100 µl/disc) and sulfur-6 showed zones of inhibition against *E. coli* O157:H7 (12.67±0.31/50 µl/disc and 20.13±0.30/100 µl/disc), *Shigella dysenterae* (10.33±0.20/50 µl/disc and 22.33±0.18/100 µl/disc), *Salmonella typhi (*14.33±0.28/50 µl/disc and 21.67±0.35/100 µl/disc), and *Vibrio cholera* (9.13±0.20/50 µl/disc and 18.05±0.08/100 µl/disc). MIC and MBC were done by broth dilution method where Merc-sol-6 displayed a strong antimicrobial activity with MIC and MBC values as low as 64 µl/ ml and 128

µl/ml, respectively against *E. coli* O157:H7 , *Salmonella typhi* where 226 µl/ ml and 512 µl/ml, respectively against *Shigella* dysenterae and *Vibrio cholerae,* on the other hand sulfur-6 displayed a strong antimicrobial activity with MIC and MBC values as low as 64 µl/ ml and 128 µl/ml, respectively against *E. coli* O157:H7 and *Salmonella typhi* and 226 µl/ ml and 512 µl/ml against *Shigella* dysenterae and 128 µl/ml and 256 µl/ml against *Vibrio cholerae.* Merc-Sol-6 and Sulfur-6 were Potentized/Dynamized to Merc-Sol-7 and Sulfur-7 and their antibacterial activity was determined. Merc-Sol-7 showed zones of inhibition against *E. coli* O157:H7 (9.40±0.10/50 µl/disc and 11.04±0.01/100 µl/disc), *Shigella dysenterae* (8.5±0.34/50 µl/disc and 10.25±0.06/100 µl/disc), *Salmonella typhi (*8.23±0.12/50 µl/disc and 10.13±0.31/100 µl/disc),*Vibrio cholera* (9.34±0.12/50 µl/disc and 10.21±0.20/100 µl/disc) and Sulfur-7 showed zones of inhibition against *E. coli* O157:H7 (9.37±0.01/50 µl/disc and 11.04±0.40/100 µl/disc), *Shigella dysenterae* (8.35±0.02/50 µl/disc and 10.34±0.08/100 µl/disc), *Salmonella typhi (*8.33±0.08/50 µl/disc and 9.38±0.06/100 µl/disc),*Vibrio cholera* (8.23±0.20/50 µl/disc and 11.05±0.08/100 µl/disc). These findings showed a little less activity than merc-sol-6 (*E. coli* O157:H7; 45.05%, *Shigella dysenterae;* 33.66%, *Salmonella typhi;* 47.05%, *Vibrio cholera;* 47.53%) and sulfur-6 (*E. coli* O157:H7; 45.16%, *Shigella dysenterae;* 53.74%, *Salmonella typhi;* 56.76%, *Vibrio cholera;* 38.78%), suggesting that activity of dynamized homoeopathy medicines sometime for some reasons would not be increased. Bio-potency of Merc-Sol-6 and Sulfur-6 were determined in comparison to Azithromycin. Bio-potency of Merc-Sol-6 showed 19.33%, 20.42%, 8.96%, 23.16%, and, 17.84% against *M. luteus, E. coli* O157:H7, *Shigella dysenterae, Salmonella typhi,* and *Vibrio cholerae,* respectively and Sulfur-6 showed 20.56%, 39.92%, 23.52%, 23.59% and 20.88%, against *M. luteus*, *E. coli* O157:H7, *Shigella dysenterae, Salmonella typhi*, and *Vibrio cholerae*, respectively. Comparative study of Merc-Sol-6 and Sulfur-6 to Azithromycin was carried out which showed 80.33%, 66.08%, 86.52%, and, 78.42 against *E. coli* O157:H7, *Shigella dysenterae, Salmonella typhi,* and *Vibrio cholerae*, respectively and Sulfur-6 showed 91.31%, 85.17%, 91.11%, and 81.67%, against *E. coli* O157:H7, *Shigella dysenterae, Salmonella typhi,* and *Vibrio cholerae,* respectively. From this investigation it is suggested that Merc-Sol-6 and Sulfur-6 can be used in controlling infectious diseases caused by *E. coli* O157:H7, *Shigella dysentrae*, *Salmonella typhi*, and *Vibrio cholera*. Extensive researches are to be necessary to find any other homoeopathy medicines against active against enteric and non-enteric human infectious diseases.

CHAPTER: 01
INTRODUCTION

1. Introduction

Homoeopathy is a system of medicine that has been used across the world for more than 200 years which heals illness using substances capable of causing the same illness—that is, presenting the same symptoms, syndrome, and conditions—when administered to healthy people.[1] Homoeopathy is founded on the principle of assisting the body in its own adaptive, healing response to symptoms and illness.[2]The homeopathic system consists of specific, highly diluted homeopathic medicines, or remedies, as well as specific treatment contexts for their uses. It is controversial and clouded by diverse beliefs and opinions.

Although homoeopathy and allopathic medicine can be, and often are, successfully used together, a few of the principles and actions of homoeopathy challenge basic concepts to allopathic medical philosophy and science.

To truly understand the value of homoeopathy, study and learning are just the beginning: observing homoeopathy used in the context of professional treatment allows for a deeper view of its efficacy and its essence. Indeed, seeing how Western-trained health care practitioners successfully use homoeopathy in their practices is vital to a professional appreciation of it. Knowing where and how homoeopathy fits, as well as where it does not fit, into comprehensive, patient-centered medical care is essential to its application and acceptance. For pharmacists, understanding homoeopathy can be important for 2 reasons: communication with patient increases and overall health outcomes improve.

Etymologically, homoeopathy is derived from the Greek words for "similar" (homoios) and "suffering" (pathos). Its system is based on thelaw of similars, which states that elements or substances that, in large doses, can create illness can, in minute doses, stimulate the body's nonspecific adaptive healing response.[3]Homeopathic medicines are derived from raw plant, mineral, and biologic materials that are specified according to monographs of the Homeopathic Pharmacopeia of the United States (HPUS), which is the nation's official compendium for homeopathic drugs.[1]

Examples of plants made into homeopathic medicines include *Arnica montana*, *Allium cepa* (onion), *Ledum palustre* (wild rosemary), *Hypericum perforatum* (St. John's wort), *Ruta graveolens* (rue), and *Bellis perennis* (daisy). Examples of minerals made into homeopathic medicines include *natrum muriaticum* (sodium chloride), *calcarea carbonica* (calcium carbonate), *kali bichromicum* (potassium dichromate), and *magnesia phosphorica* (magnesium phosphate). Examples of biologic materials made into homeopathic medicines

include *Apis mellifica* (honeybee), *Anas barbariae* (duck extract), and *Lachesis mutus*(Bushmaster venom).[4] Although some of these materials are also used in other forms of nonconventional therapies, such as nutritional supplements and Western and Chinese botanical treatments, homeopathic medicine uses extremely low doses of these substances to stimulate a healing response.[5,6]

Symptoms and their specificities are central to homoeopathy. It is vital that presenting symptoms not require immediate medical attention because homoeopathy is never intended to substitute for or delay urgent care: homoeopathy is best used for non-urgent, self-limiting conditions. Symptoms of self-limiting conditions are matched to the indicated homeopathic medicine. This matching is aided by consultation with a trained pharmacist, pharmacy technician, or homeopathic practitioner. Labels on homeopathic products can be helpful, but they are often limited in scope, listing only 1 or 2 general symptoms. Optimal recommendation of homeopathic medicines typically calls for more than 1 symptom to be reported or more specific information to be known. For example, numerous remedies may be used for the treatment of warts; knowing which remedy is best requires more information about the condition, as well as familiarity with commonly used remedies. [5,6,7,8,]

The following simple example highlights how symptoms and homeopathic medicines are matched. When a healthy person slices an onion, he or she typically experiences clear burning tears and a clear runny nose that stings the face; these symptoms improve with fresh air. A person with mild hay fever symptoms experiences clear, but irritating, runny eyes and nose that improve with fresh air. Therefore, according to principles of homeopathic medicine, a microdose of *Allium cepa* (onion) would help the body resolve symptoms of hay fever. This is an example of how *like cures like*. [5,6]

However, because homoeopathy is highly individualized, 2 patients seeking hay fever relief might require different medicines, depending on their unique and precise symptoms. In the first example, nasal secretions were irritating, so the pharmacist would recommend *Allium cepa*. If a second patient presented with clear tears that burned and clear non-burningnasal discharge, the pharmacist might recommend a homeopathic form of *Euphrasia officinalis* (eyebright). The closer the symptoms match the preparation's unique and specific characteristics, the better the chance for stimulating or engaging the body's own innate healing forces.[5,6,9]

In addition to the condition's specific and general symptoms, location on the body, contributing causes, etiology, and onset, a variety of other individual factors, or modalities,

should be considered when choosing a homeopathic remedy. A trained homeopathic practitioner will ask the patient many specific questions, including whether the symptoms are better or worse at certain times of day, at rest or in motion, or with warmth or cold and whether there are any other accompanying physical, emotional, or psychological discomforts.[5,6]

1.1. Clinical example

A 55-year-old woman presents to her community pharmacy to seek a low-cost treatment for her osteoarthritis pain that will not cause side effects or interactions with her other medications. She visits her doctor regularly and wants to minimize ibuprofen and acetaminophen use. She finds 2 different single (microdose) homeopathic medicines for arthritis: *Rhus toxicodendron* (poison ivy) and *Bryonia alba* (white bryonia). Upon questioning, she tells the pharmacist that her stiffness is worse in the morning, gets better as she moves around, and improves with a hot shower. These symptoms fit the symptom profile for *Rhustoxicodendron*; in comparison, *Bryonia alba* would be recommended if she felt better when resting and when applying cold compresses. The pharmacist should instruct the patient to take the *Rhus toxicodendron* pellets under the tongue, or dissolved in the mouth, on an empty stomach. They should be taken frequently at the onset of pain, and the frequency of administration can be decreased to an as-needed basis when symptoms improve. In this way, homeopathic medicines are often dosed differently than over-the-counter (OTC) or prescription medications: once a patient experiences substantial relief, he or she should discontinue the homeopathic regimen and reinstate it only if symptoms return. If appropriate for the patient, homeopathic medicines may be taken concurrently with conventional arthritis medications.[10]

If homeopathic practitioners able to convince and understand patients and develop patient's trust on homeopathic medicines, this encourages patients to fully disclose all the medications and products they are taking, which, in turn, helps homeopathic practitioners/pharmacy staff members, as well as other health care professionals, offer better and more complete care.

A background in chemistry and pharmacy offers a little context to begin to understand homoeopathy and other systems outside of conventional medicine. However, patients are increasingly asking questions about the homeopathic system and offering personal reports of its use, regardless of homeopathic practitioners /pharmacists' comfort levels with its

principles and remedies. Homeopathic practitioners should work to gain an appreciation for the history, concepts, practice, advantages, and shortcomings of this treatment model. Homoeopathy is a complex system of medicine, with the power to help many common conditions if appropriately understood and recommended. It takes homeopathic practitioners many years (even decades) of intensive study and practice to learn to treat long-standing or chronic health conditions. For this reason, it is often reasonable to refer patients to expert practitioners of this discipline for conditions that are chronic in nature or if patients do not experience adequate relief from simple OTC recommendations.[11]

Homeopathic medicines for self-limiting conditions have very little chance of doing any harm. Because homeopathic medicines are used in highly diluted doses, they do not interact or interfere with the pharmacokinetics or pharmacodynamics of conventional drugs and, therefore, homeopathic and allopathic medicines can be used together. Further, homeopathic medicines do not cover up or mask medical emergencies or serious conditions. They are not intended to delay or replace conventional medical treatment. In fact, when patient conversations begin with a question about homoeopathy, pharmacists have an opportunity to encourage consultation at the pharmacy or to provide referrals to appropriate clinicians. Today, homoeopathy is a part of every pharmacist's practice and it is one of the many options available for advising patients about self-limiting conditions.[11]

1.2. History and applications of homoeopathy

In the early 19th century, Samuel Hahnemann, a German physician, developed the homeopathic system of medicine based on the principle of *like cures like* cited by Hippocrates and Paracelsus and used in ancient cultures across the world.[17(2)] According to biographer Richard Haehl, Hahnemann's interest in the law of similars was sparked while translating a book by leading Scottish physician and chemist William Cullen, who held that cinchona bark was effective for treating malaria because it was bitter and astringent. Hahnemann knew that other substances with these particular properties were not effective for malaria treatment, so he decided to investigate Cullen's claim firsthand. He began experimenting with cinchona, eventually taking a large dose himself, which, in turn, induced malaria-like symptoms. He later concluded—and verified through extensive experimentation with this and other substances—that the bark was an effective treatment because it caused symptoms similar to the disease it was treating.[12]

Hahnemann spent the next 6 years experimenting and writing about his success with this and other substances and meticulously and systematically documenting his findings. Hahnemann gained significant notoriety and was very successful in treating numerous conditions, particularly infectious diseases and epidemics, utilizing the law of similars and his homeopathic preparations.[27(12)] The standard medicines of his day were often highly toxic and included leeches and bloodletting, so the practice of what Hahnemann would ultimately call homoeopathy gained a large following in Europe, Asia, and the United States (U.S.). In 1844, the American Institute of Homoeopathy was founded; it was the first national medical society in the U.S. and it remains in existence to this day.[13]

1.3. Homoeopathy in the U.S.

The popularity of homoeopathy continued to grow throughout Europe and in many parts of Asia. However, in the U.S., the rise of new technologies and pharmaceuticals during the early 1900s led to a decline in the interest and practice of many traditional therapies, including homoeopathy. Recently, the U.S. has faced growing crises of antibiotic overuse and drug toxicity and interactions. Now, patients and providers are once again considering options that avoid side effects and support the body's own natural healing abilities. According to the U.S. National Institutes of Health (NIH), approximately 38% of adults (3.9 million) and 12% of children were using some form of and alternative medicine (CAM) in 2012. These results from the National Health Interview Survey were similar to the findings from 2007.[14,15] The survey also noted that, while people of all backgrounds use CAM, its use is greater among women and among people with higher levels of education and income.[14]

1.4. Applications of homoeopathy

Homoeopathy is used as self-care for a variety of conditions, as well as for management of both acute and chronic conditions through health care practitioners. Common acute physical and emotional conditions that respond well to homeopathic medicines include.[16,17]

- Allergic rhinitis
- Stress and anxiety
- Common cold
- Sore throat
- Flu symptoms
- Postsurgical pain, swelling, and bruising

- Motion sickness
- Insomnia
- Premenstrual syndrome
- Menopausal vasomotor symptoms
- Digestive discomforts
- Warts
- Conjunctivitis
- Insect bites
- Minor sunburn
- Minor skin irritations
- Muscle and joint aches
- Sprains, strains, and minor traumas

The best applications for OTC homeopathic treatment are self-limiting situations. Again, OTC homoeopathy should not be used in place of conventional medical care that is indicated for a serious condition.

Homeopathic preparations are available as single medicines (i.e., 1 active ingredient) or as branded medicines (i.e., often a combination of active ingredients). The delivery forms of homeopathic remedies are various and also common to conventional drugs:

- Sublingual or self-dissolving (pellets, tablets, and liquids)
- Topical (ointments, creams, lotions, gels, and sprays)
- Ophthalmic drops
- Otic drops
- Nasal sprays
- Vaginal and rectal (suppositories, creams, and ointments)

1.5. Principles of homoeopathy

In contrast to allopathic medicine, which often focuses on suppressing or palliating symptoms, homoeopathy's overarching goal is to stimulate and support the body's own inherent healing defenses. The treatment focus and underlying principles are, thus, fundamentally different from conventional medicine. True understanding of homoeopathy requires both consideration of the homeopathic principles on their own and practical experience in using them with patients.

1.6. "Like cures like"

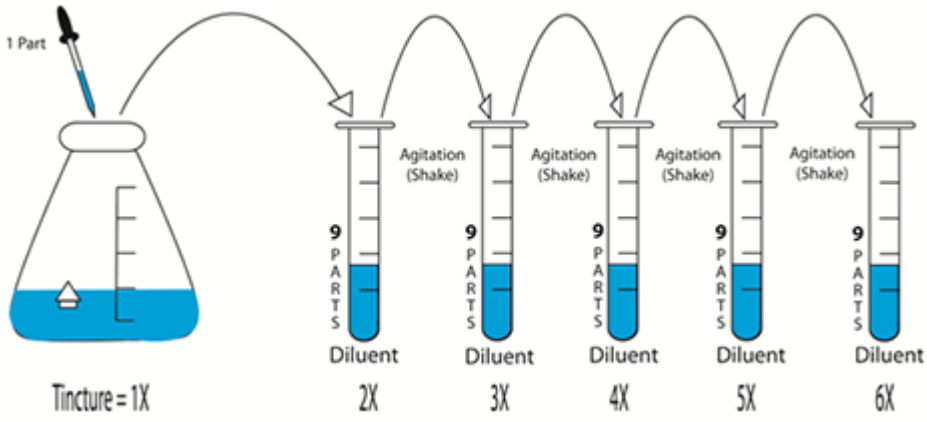
The first key principle in homoeopathy is the *law of similars*, which holds that *like cures like*.[18] That is, a substance that, in its crude form, produces certain symptoms in healthy people can cure the same symptoms in the sick when prepared in a homeopathic dose.

For example, when consumed in large amounts, coffee can cause a stimulated, active state that might result in difficulty sleeping. The low-dose homeopathic medicine made from green coffee, *Coffea cruda*, can be used for insomnia characterized by sleeplessness caused by mental hyperactivity.[19]While such an example seems illogical, pharmacists and pharmacy technicians are accustomed to paradoxical dose-related responses. For example, some pharmaceuticals, such as chemotherapy agents, are used therapeutically to treat cancer, but high doses of these agents can result in therapy-related toxicity and malignancies.[20]

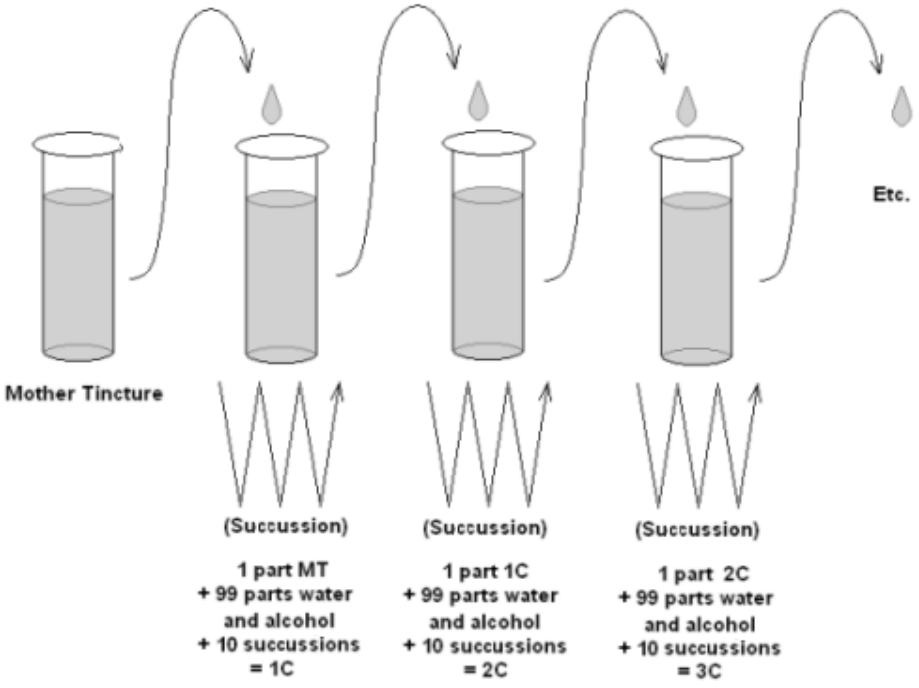
1.7. Minimum dose

In homoeopathy, the minimum dose principle holds that only the very minimum amount of medication should be given to elicit a response.[18]This is the rationale for using highly diluted and vigorously shaken potencies of homeopathic medications. As Figure 1 shows, these medicines are prepared according to specifications by their degree of dilution: X potencies are factors of 10 (decimal) and C potencies are factors of 100 (centesimal). The roman numerals denote the factor by which the substance has been diluted (X is the process of dilution by a factor of 1:9; C is the process of dilution by a factor of 1:99, Fig.1).[21]

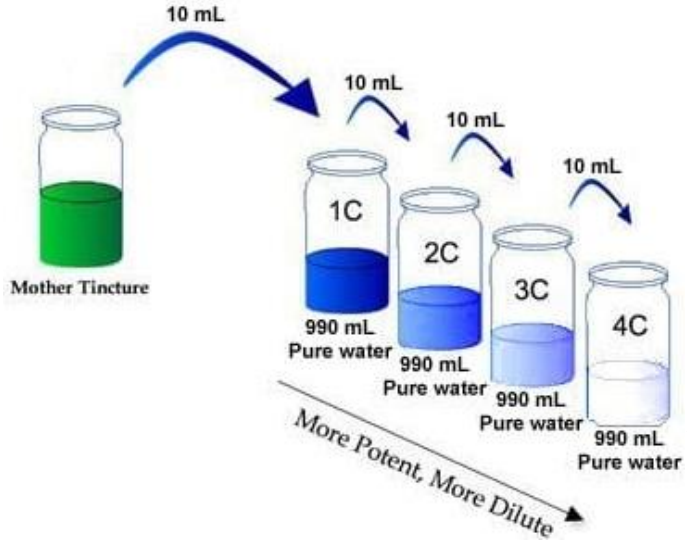
(a) The Homeopathic Dilution Process



(b) The Homeopathic Dilution Process
Centesimal (C) Scale



or



tional Repository

Figure 1. Homeopathic medicine dilution.

(a) X potency medicines are diluted by factors of 10.
(b) C potency medicines are diluted by factors of 100.

A homeopathic medicine is made with carefully delineated and controlled successive homeopathic dilutions, each followed by precisely directed succussion. For C potencies, the process begins by taking 1 part of 1C potency and adding it to 99 parts diluent (water or alcohol), followed by succussion, which creates the 2C potency; then 1 part of 2C is added to 99 parts diluent to make 3C. This process continues to make the common C-potencies used in community pharmacy practice: 6C, 9C, 15C, and 30C.[21]

The concepts of potency and dilution are important for pharmacists and pharmacy technicians to understand: homeopathic medicines are sufficiently dilute to encourage innate healing responses and yet pose little risk of side effects or pharmaceutical interactions when used at the doses described above. [22,23]

1.8. Single remedy

The single remedy principle states that 1 homeopathic medicine should be prescribed at a time—that is, the single medicine that most closely matches the totality of the patient's symptom complex or picture should be used. The trained homeopathic practitioner uses this classic or constitutional approach and views the overall symptom picture holistically rather than as symptoms occurring from separate disharmonies.[24]There are, however, numerous OTC branded combination formulas of homeopathic remedies. The active homeopathic medicines contained within these preparations are carefully selected on the basis of each individual medicine's symptom profile and the most commonly indicated use of each. Since it is often difficult to quickly ascertain which would be the single most effective homeopathic medicine during a brief consultation, the goal of these combination formulas is to ease the process of remedy selection. This is particularly true for the topical combination formulas designed to treat pain and for the oral formulas designed to treat colds and cough, hay fever, allergies, motion sickness, ear or teething pain, and restless legs. The general rule of thumb,

according to highly trained homeopathic practitioners, is that the single most indicated remedy will offer the fastest, greatest, and most lasting therapeutic benefit. But again, this is difficult to determine without having advanced experience and knowledge and spending the necessary time with the patient.[25]

In addition to the 3 homeopathic principles already described, the treatment context is an essential component of homoeopathy. A caring practitioner is key to this context. Pharmacy practice is about more than dispensing medicines—it is also about the manner with which medicines are dispensed. When practitioner/pharmacists show kindness and interest in patients, patients typically feel better and more relaxed, which makes it easier to discuss their symptoms and all available treatment alternatives. This, in turn, builds trust. In general, the more pharmacists know about different therapies, the more patients will tell them and the better able pharmacists are to do the job of caring for patients.

This communication focus was the emphasis of the NIH's *Time to Talk* program—a campaign focused on creating more dialogue about CAM among patients and their health care providers to facilitate better health management and decision-making across all therapeutic options.[26]

1.9. Status of homoeopathy research

Securing research funding and conducting research are challenging for every medical discipline today, but the nature and underlying philosophies of homoeopathy offer particular obstacles to research. There are, however, numerous studies that are noteworthy demonstrations of the efficacy and safety of homoeopathy. A brief review of this literature and research of plausible homeopathic mechanisms of action are presented below.

1.9.1. Basic research

Basic scientific research in homoeopathy focuses on biological and physical evaluations of dilutions, including their actions and their potential mechanisms of action. Not surprisingly, scientific skepticism about homoeopathy focuses on its use of these very high dilutions, including ultra-molecular dilutions, in which there are no longer molecules of the starting substance present.[27]

Several emerging areas of research are investigating possible mechanisms of action of homeopathic dilutions.[28] One theory is that the structure of the solvent molecule (water or alcohol) might be imprinted with the vibratory properties of the original tincture.[29] Other research areas include examinations of nanoparticles[30] and the effects homoeopathy has on

immune modulating, cell signaling molecules,[31] and innate hormetic, adaptation, and nanoparticle mechanisms.[32]

The Homeopathic Basic Research Experiments database (HomBRex) contains information about basic research experiments on homoeopathy, including the effects of homeopathic preparations in bioassays and nuclear magnetic resonance spectroscopy, as well as the physico-chemical effects of the preparation process.[33] The nature of these studies is highly complex and, therefore, not well-disseminated or even discussed within most mainstream pharmacy or medical circles.

1.9.2. Clinical research

A key challenge in clinical research in homoeopathy is that homeopathic medicines are designed for individual expressions of a disease, rather than a disease or single symptom, which increases the complexity of randomized controlled trials (RCTs). Nonetheless, several clinical and epidemiological studies have been conducted that yielded substantial positive results. For example, a 2013 study tested the efficacy of a homeopathic syrup for treating cough arising from upper respiratory tract infection with a randomized, double-blind, placebo-controlled clinical trial. Cough scores decreased in both groups over time, but, after 4 and 7 days of treatment, cough severity was significantly lower in the homeopathic group (P< 0.001) than in the placebo group (P = 0.023).[34]

Increasingly, researchers are designing studies that respect homeopathic principles of individualized treatment. A study on fibromyalgia, for example, demonstrated that individualized homoeopathy is substantially better than placebo for lessening tender point pain and improving quality of life and global health.[35]

In the side of infectious diseases, treatment with homeopathic medicines has faced tremendous challenges, because of unavailable experimental evidences in most of the cases of antimicrobial activities. But some investigators had studied *invitro* with some homeopathic medicines against human pathogens, for example, a study indicated that *Boerrhavia diffusa* mother tincture had excellent activity against *Escherichia coli*. Mother tincture of *Chorozophora plicata* showed highly effective results against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, and *Echinops echinatus* mother tincture showed highly effectiveness against *Salmonella typhi*. *Heliotropium europaeum* mother tincture exhibited highly effective results against *Bacillus subtilis*. *Tamrix aphylla* presented

activity against *Bacillus subtilis*.[36] Two homeopathic tinctures such as *Arsenicum album* (mineral extract) and *Lycopodium clavatum* (plant extract) have antibacterial activity (*in vitro*) against the periodontal bacteria: *Actinomyces israelii*, *Streptococcus sanguinis*, *Prevotella intermedia*, and *Phorphyromonas gingivalis*.[37]. In another study, ***i****n vitro* growth of *staphylococcus aureus* exhibited singnificant reduction in the presence of *Mercurius solubilis* 6c, 12c, 30c, 200c, 1M, 10M, 50M[38]. *Commiphora molmol*, *Inula helenium*, and *Thymus vulgaris* showed antimicrobial activity against *E. coli 0157:H7*, *S. aureus* and *Enterococcus hirae*, and over the entire dilution range (10^{-1} to 10^{-7}).[39] In vitro growth of MRSA exhibited statistically significant reduction in the presence of *Belladonna*, nosode 6 CH and 30 CH, Hepar sulfur 30 CH and Silicea 6 CH compared to 30% alcohol ($p< 0.0001$).[40]

1.9.3. Research models

To understand homeopathic research and its critics, it is necessary to appreciate the assumptions of the homeopathic model. To this end, some researchers are developing validity models for RCTs that aim to identify relevant judgmental domains to use in assessing the validity of the homeopathic treatment model.[36]The need for individualized therapy and the lack of clearly defined clinical outcome parameters are current challenges to RCTs investigating homeopathic medicine.

1.10. Barriers to homoeopathy use and practice

A significant barrier to homeopathic use by the public, health care practitioners, and scientists, alike, is the seemingly paradoxical concept that ultra-small doses are capable of producing clinical effects. This is an area of important, but still nascent, emerging research. For this reason, most pharmacists and pharmacy technicians as well as most medical professionals understand little to nothing about the principles or practice of homeopathic medicine and do not, therefore, recommend its use. Many other barriers to use stem from misconceptions and misunderstandings about homoeopathy.[22,42]

1.11. Myths and facts of homoeopathy

Several misconceptions about homoeopathy prevent its widespread use and acceptance. Below, common myths about homoeopathy and homeopathic practice are explained:[43-45]

- *Homeopathic medicines take a long time to work*. The effect of a homeopathic medicine may be rapid (minutes to hours), or 1 or more days may be needed for its full effect. The time required for symptom relief is nonuniform because the medicines do not create the response—rather, the medicines stimulate the body's own secondary healing response. If properly prescribed for an acute self-limiting condition, homeopathic medicines can work very quickly, but effective treatment of long-standing, chronic ailments may take days to several weeks. Typically, if no symptom relief occurs within 1 to 2 weeks (for chronic ailments), a different remedy should be recommended or the patient should be referred to a specialist.
- *Homeopathic medicines are difficult to ingest*. Patient compliance is an issue with medicines of any type, but some people assume that compliance is particularly difficult with homeopathic medicines because the sublingual or buccal delivery form means giving up eating certain foods and drinking coffee. This is not always the case, though, homeopathic practitioners' opinions on the matter vary. For better buccal absorption, it is generally suggested that homeopathic medicines be taken with a clean mouth, away from strong flavors (e.g., strong mint or menthol products); they are typically taken on an empty stomach, unless otherwise indicated. Some practitioners also suggest that the medicines be taken without touching the pellet or tablet: patients can use the cap to place the medicine under the tongue or onto the buccal mucosa, where it is allowed to slowly dissolve.
- *If homoeopathy can cure many things, it should cure everything*. Homoeopathy is limited by the body's ability to respond to homeopathic stimulation. If a condition has progressed too far or the immune system is compromised by drugs or another condition, the body may not be able to mount an appropriate immune response. However, even in these cases, homeopathic medicines could still be considered for concurrent self-limiting symptoms.
- *Homeopathic outcomes are only a result of the placebo effect*. Some studies of homoeopathy do show similar outcomes to placebo. However, numerous other clinical research studies have compared homeopathic medicines with placebo and found substantial differences in the actions, durations of activity, and outcomes.

1.12. Patient awareness

Another common barrier to homoeopathy use among pharmacists and patients is primarily related to a limited education and knowledge-base about homoeopathy. Many patients assume, for example, that homeopathic medicines are not subject to government regulation, while, in fact, they are regulated by the U.S. Food and Drug Administration (FDA) and manufactured according to precise industry standards.

Terminology and general principles of administration offer other issues and barriers. For example, patients may not understand what *sublingual* means (i.e., letting a pellet or tablet dissolve under the tongue rather than chewing it). They may also be concerned that a homeopathic medicine might have interactions with other prescriptions or natural products they are taking. Further, patients may assume that because they did not achieve any benefits from a previous trial of a homeopathic medicine that the system as a whole will not work.

Finally, many patients assume that pharmacists and pharmacy technicians have little to no knowledge of homoeopathy. This underscores the importance of the need for all health care professionals to learn about all systems and methods of care in order to initiate and participate in discussions with patients and serve as reliable, reputable sources of information.[46]

1.13. Physician and pharmacist education

In the U.S., homoeopathy is not routinely part of the curriculum for health care providers. Many pharmacy professionals know very little about homoeopathy, and this module may be the most comprehensive source of information on the subject that many have studied. Likewise, few physicians and pharmacists realize that homeopathic medicines are OTC products that are FDA regulated and manufactured under strict pharmaceutical guidelines.[47,48] Naturopathic schools of medicine are an exception to this lack of education: homoeopathy is an integral part of the curriculum and scope of naturopathic practice.[49] Other health care practitioners can obtain continuing education and training in clinical homoeopathy from various groups.

1.14. Perceptions about efficacy and clinical trials

The concept of homeopathic dilution is counterintuitive. Typically, conventional medicines are made stronger by adding more ingredients, but, in homoeopathy, a higher potency is often, although not exclusively, one that is more diluted and succussed.

Similarly, the concept of the "strength" of a homeopathic medicine is counterintuitive: sometimes less is more. Efficacy is facilitated by the appropriateness of the dilution to the patient's symptoms, not the amount of active ingredient in a formulation.

The purpose of a homeopathic medicine is to encourage the body's inherent defenses to correct imbalances. Sometimes that reminder is solely on a molecular (not a material) level. Understandably, this concept (i.e., ultradilution or nanopharmacology) creates a substantial barrier for people trained in traditional chemistry or conventional medicine. The process of coming to understand a nonmaterial effect is something that may only occur through engagement with and experience in the art and practice of homoeopathy. [50,51]

The science of homoeopathy is complicated and it is still emerging. As explained, clinical studies have shown mixed results, particularly when therapeutic approaches are not individualized. However, when studies account for the full context of treatment—that is, the medicines, the discussions about symptoms and modalities, and the caring provider—they show positive effects of homeopathic medicines that surpass those of placebo alone and offer no serious adverse or toxic effects.[50,51]

1.15. Safety concerns

No form of medicine is completely safe for all patients all the time, but homoeopathy can be much safer than many conventional and herbal medicines—a fact that seems foreign to many health care professionals. Because homeopathic medicines are administered in the smallest possible microdoses, there is almost no chance that they will exert any pharmacokinetic effect in terms of absorption, distribution, metabolism, or excretion. So, while an allopathic drug might interfere with a person's ability to respond to a homeopathic medicine, a homeopathic medicine will likely not interfere with an allopathic drug's effect. This is may not always be true of conventional treatments.[52,53]

A key benefit of homeopathic medicines is that they can alleviate symptoms without masking conditions. If, for example, a person was having severe abdominal pain, homoeopathy would not be indicated. However, even if that patient took a homeopathic formula for the pain, it would not mask the underlying disease process or any diagnostic symptoms in a true medical situation. In contrast, taking a pharmaceutical pain medication might reduce the pain and, thereby, delay a medical response. Similarly, steroids are administered to reduce inflammation, yet they allow the inflammatory disease processes to continue. Homeopathic medicines can treat symptoms, such as pain and inflammation, without masking critical

symptoms or interfering with a diagnosis. This is an invaluable piece of information to consider.[54]

Chemical sensitivities are a consideration for all products in the pharmacy. Patients are sometimes confused about additional ingredients added to homeopathic preparations, such as alcohol, lactose, and sucrose. For example, a patient with lactose intolerance may wish to avoid a product that uses lactose as an additive. However, the amount of lactose in homeopathic pellets and tablets is usually far below the threshold of discomfort; research suggests that adults and adolescents with lactose malabsorption can eat or drink at least 12 grams of lactose in one sitting with only minor, if any, symptoms. Still, in rare cases of complete abstinence or true allergy, such bases and additives must be avoided. Further, pharmacists must be respectful of patients' needs, preferences, and practitioner instructions and direct patients to the appropriate products in the case of ingredient sensitivity.[55]

1.16. Clinical examples

A 35-year-old man presents to the pharmacy. He states that he will be having minor outpatient surgery next week and he enquires about pain relief. He typically uses Arnica gel for joint pain after he exercises and he wonders if he can use it after the surgery. Overall, he is in good health, and his only current medications are atorvastatin 20 mg daily, a daily multivitamin, and a daily antioxidant supplement that he purchases elsewhere. The pharmacist tells him that homeopathic dilutions of *Arnica montana* (mountain daisy) can be taken sublingually to reduce pain, swelling, and the discoloration of bruising. He should allow the pellets to dissolve slowly in his mouth or under his tongue. The medication should be taken on an empty stomach and without strong flavors (e.g., mint and menthol); it should be taken every few hours initially, and he can decrease the frequency as his symptoms improve. Arnica gel should NOT be applied to an open wound. Although Arnica preparations can be taken safely with his medications, he should tell his doctors about all the supplements he takes because they may need to be discontinued before surgery. The homeopathic medication should be stopped completely when he is recovered.[45]

1.17. Regulatory issues in homoeopathy

It is paramount that pharmacists and technicians understand the regulation of homeopathic medications. Not only is this area of confusion leading to widespread misinformation, it is an area of rapid change. Pharmacists are dedicated to providing accurate drug-related

information and, in this capacity, should combat the misconception among the public and other health care professionals that homeopathic medicines are not regulated. The most current FDA distinctions regarding how products—including homeopathic medicines—are regulated, approved, and advertised for conditions and indications are described below.[47]

1.18. Regulation of medicines and supplements

Pharmaceutical and homeopathic medicines, both prescription and OTC products, are regulated differently than dietary supplements.[51] Dietary supplements include vitamins (e.g., folic acid, ascorbic acid), minerals (e.g., calcium, magnesium), herbs (e.g., ginger, turmeric, and other botanicals), amino acids (e.g., l-lysine, tryptophan), probiotics (e.g., *Lactobacillus*, *Bifidobacterium*), fish oils, and other nutrient components.[57]

Conventional prescription drugs are Food and Drug Administration (FDA) approved for a specific condition and must obtain pre-market approval; they are also subject to post-market surveillance. Dietary supplements are under the jurisdiction of the Dietary Supplement Health and Education Act of 1994 (DSHEA), which does not categorize them as drugs, and, therefore, requires no pre-market approval. As a result, manufacturers are prohibited from making disease claims on the labeling for dietary supplements.[58,56]

However, DSHEA does allow the manufacturers of dietary supplements to make structure/function claims, which specify how the ingredients maintain normal physiological structure or function or contribute to well-being. Examples of structure/function claims include the following: "maintains cell integrity," "maintains bowel regularity," and "builds strong bones." If such a claim is made, however, the following disclaimer must also be included on the supplement label: "The FDA has not evaluated this claim." The disclaimer must also state that the product is not intended to "diagnose, treat, cure, or prevent any disease" because only a drug can legally make such a claim.[59] Again, because homeopathic medicines are not dietary supplements, they do not necessitate the use of this disclaimer.

1.19. Regulation and manufacturing of homeopathic medications

In the U.S., homeopathic medicines are regulated as drugs under the Federal Food, Drug, and Cosmetic Act (FDCA). The FDA regulates homeopathic product compliance related to manufacturing and labeling, and the Federal Trade Commission (FTC) regulates advertising.[47,60]

The FDCA recognizes the HPUS as the authority for homeopathic medicine manufacturing, which must be performed in accordance with strict HPUS guidelines.[1(47)] The FDA regularly inspects manufacturing organizations and their facilities, which must comply with all current Good Manufacturing Practices under the FDA's Code of Federal Regulations Title 21, Part 210.[61] The HPUS is the official compendium recognized by the FDA for homeopathic drugs in the U.S. and currently standardizes more than 1000 homeopathic drugs.[62]

In April 2015, the FDA held a 2-day public hearing focused on its regulation of and labeling requirements for OTC homeopathic products. The hearings featured representatives from the public, professors, lawyers, and regulators, as well as the manufacturers of homeopathic products, pharmacists, educators, public policy officials, medical doctors, naturopathic doctors, and other practitioners.[63]There was overwhelming testimony from speakers in consistent support of homoeopathy and existing FDA regulations for these remedies with very little dissension. Supporters disclosed that homeopathic remedies offer benefits of decreased cost and increased safety, but critics spoke against homoeopathy, citing concerns such as its use preventing patients from seeking medical care. However, the general consensus among advocates and skeptics, alike, is that homeopathic medicines are fundamentally safe. The intent of these testimonies was to help improve consumer protections through review of Good Manufacturing Practices while aiming to maintain an optimal amount of freedom for the individual to access safe medicines.[64,65]

It is vital that pharmacists and technicians understand how to interpret safety and dangers of all medications, including homeopathic medications. Although homeopathic medicines have an outstanding safety profile, absolute safety is not guaranteed. The homeopathic pharmaceutical industry adheres to rigorous standards and monitors the occurrence of adverse events, just like the traditional pharmaceutical industry. Industry data indicate that, while rates are extremely low, there are rare incidences of adverse events and serious adverse events associated with the use of homeopathic medicines.[66]When dangers related to homeopathic remedies are apparent, the FDA issues warnings to educate the public and health care providers about the safe use of the products.

In March 2015, the FDA issued an official warning to consumers not to rely on homeopathic asthma products–particularly for relief of acute asthma symptoms. This reinforces the concept that OTC homeopathic medicines should only be used for self-limiting conditions, not for urgent medical situations such as an asthma attack.[67]

In September of 2016, the FDA issued a press release warning consumers that "homeopathic teething tablets and gels may pose a risk to infants and children.''[68] As a result, Standard Homeopathic Company discontinued the manufacture and sale of certain teething products.

In April 2017, the FDA ordered Standard Homeopathic Company to issue a nationwide recall of Hyland's Baby Teething Tablets that contained homeopathic dilutions of belladonna. An investigation for mislabeling found that the products contained "inconsistent amounts of belladonna alkaloids that may differ from the calculated amount on the products' labels."[69]

1.20. Marketing and labeling of homeopathic medicines

Homeopathic drug labels are considered a form of advertisement and, as such, the FTC has jurisdiction over their content. The FTC recently determined that efficacy statements for homeopathic products may be misleading. In November 2016, the FTC took action to clarify the level of scientific proof of marketing claims on labels. The FTC stated that a product's claim can no longer be "based only on the theories of homoeopathy from the 1700s that are not accepted by most modern medical experts. To be non-misleading, the product and the claims must also comply with the requirements for homeopathic products and traditional homeopathic principles."[60]

For most OTC homeopathic drugs, the policy statement notes, "the case for efficacy is based solely on traditional homeopathic theories and there are not valid studies using current scientific methods showing the product's efficacy." Additional clarifying labeling statements will be required in order to be in compliance with the FTC policy.[60]

For the sake of all patients' health, it is imperative that pharmacy professionals stay accurately informed of the recent increase in FDA and FTC review and scrutiny of homeopathic medicines.

A glossary of terms related to homoeopathy (Table 1)[70-81] is provided for easy reference to understand homoeopathy.

Table 1. Glossary of Homoeopathy Terms

Parameters	Explanations
Adaptive response	The body's complex ability to react to changes and stresses
Allopathic medicines	Mainstream or conventional medical treatment of disease using drugs that produce opposite effects to signs and symptoms experienced by the patient.
Botanical medicines	The use of plants for medicinal purposes, generally in the form of capsules, tinctures, or powders; also called herbal medicine.

Chemical dilution	A homeopathic medicine produced by a series of dilutions of 1 part substance to 99 parts water or alcohol; referred to as a C potency
Combination remedies	Preparations made by combining 2 or more homeopathic medicines
Complementary and alternative medicine (CAM)	The U.S. National Institutes of Health defines "complementary" as the use of non-mainstream practice with conventional medicine; when non-mainstream practice is used in place of conventional medicine, it is considered "alternative"
Constitution	The overall health of the person as determined by heredity, life history, lifestyle, environment, and past treatments
Constitution al or classic homoeopathy	The method of selecting medicines on the basis of a comprehensive understanding of the whole state and nature of the patient—rather than on only 1 symptom or acute incident—followed by a waiting period to evaluate the action of the treatment
Decimal dilution	A homeopathic medicine produced by a series of dilutions of 1 part substance to 9 parts water or alcohol; referred to as an X potency
Dietary supplements	Vitamins, minerals, probiotics, amino acids, and other nutrient-related products; regulated by the Dietary Supplement Health and Education Act (DSHEA) of 1994
Dilution	A very small quantity of a natural substance in diluent
Herbal medicines	The use of plants for medicinal purposes, generally in the form of capsules, tinctures, or powders; also called botanical medicine
Homoeopathy	A system of medicine that uses diluted substances to relieve symptoms
Homeopathic medicine	Medicines based on the principles of homoeopathy that help improve symptoms by supporting a patient's own natural healing response
HPUS	Homeopathic Pharmacopeia of the United States; the official compendium for Homeopathic Drugs in the United States
Integrative medicine	Many definitions of "integrative" health care exist, but all involve bringing conventional and complementary approaches together in a coordinated way
Law of similars	The fundamental philosophy of homoeopathy, which holds that a substance that causes a set of symptoms in a healthy person acts as a curative medicine when given to a sick person with similar symptoms; also known as the principle of "like cures like"
Materia medica	Literally "materials of medicine" in Latin; refers to books that list individual homeopathic medicines and details for use
Modalities	In homoeopathy, modalities are factors that make a person's overall health or a specific symptom better or worse (e.g., for weakness that is worse in the morning or a headache made better by cold applications, the modalities would be "worse in the morning" and "better by cold applications," respectively)
Mother tincture	The first, standardized solution from which homeopathic dilutions are successively made; often designated as MT or 1X (10% original substance)
Naturopathic medicine	A distinct primary health care profession that emphasizes prevention, treatment, and optimal health through the use of therapeutic methods and substances that encourage an individual's

	inherent self-healing processe
Potency	The number of times a substance has been diluted and succussed (shaken) according to the strict rules of the Homeopathic Pharmacopeia; dilutions are expressed as decimal (X) or centesimal (C); the higher the potency, the less original substance in the finished product
Remedies	Another word for homeopathic medicines
Succussion	Part of the homeopathic manufacturing process in which a medicinal substance is diluted in distilled water or alcohol and vigorously shaken according to precise standards

The concept and even the attempt to use substances derived from one organism to kill another organisms (antibiosis) are almost as old as the science of bacteriology. (e. g. mouldy curd of Soya bean applied to boils; Fig.2).

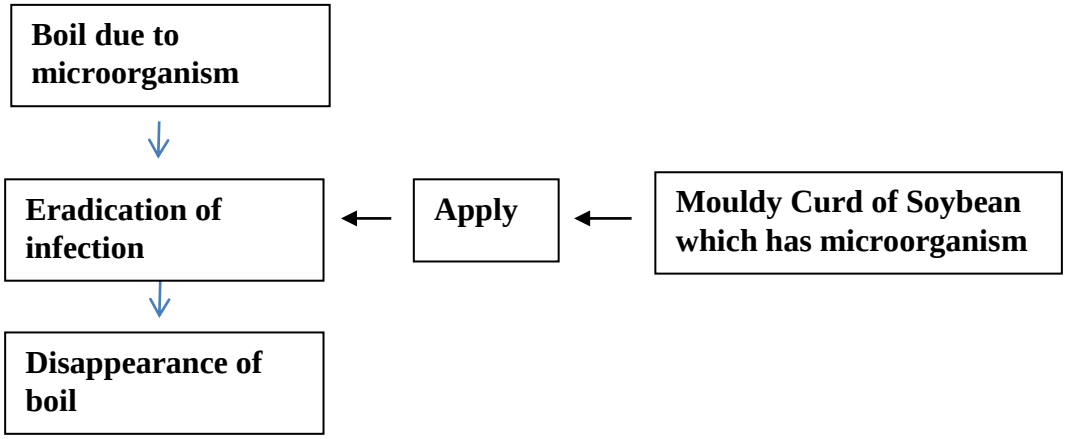


Fig 2. Antibiosis (Resembling similia similibus currentur)

This in one-way or the other resembles Homeopathic law "Similia Similibus Currenter" i. e. likes are cured by likes. The Scientists Pasteur and Joubert 1877 confirmed this, they noted that anthrax bacilli grew rapidly when inoculated into sterile urine but failed to multiply and soon died if one of the "common" bacteria of the air was introduced in urine at the same time[1]. With the breakthrough invention of the antibiotic, Penicillin, which is produced by *Penicillum notanum*, a mould, shown by Alexander Fleming in 1928, death due to infectious disease drastically, came down. Since then newer antibiotics came into the market and proved to have excellent therapeutic potential in curing the pathogenic manifestations. But amidst all this, drug resistance has become a menace for medical science. Since adaptation is quality of life and by mechanism of mutation, transformation, transduction & conjugation many bacteria have become resistant to drug.[82]It is proven beyond doubt that due to inappropriate

and unethical use of antimicrobials, apart from drug resistance, generation of new mutant forms of non-pathogenic bacteria is posing a severe threat to the medical science (e. g. Non-pathogenic *E. coli 0157:H7* has become pathogenic, once known for its symbiosis is now responsible for a wide range of GI & UI pathologies.) Now host defense mechanism is the most important determinant of therapeutic effectiveness. Therefore, stimulating ones immune mechanism would be more appropriate tool in combating the raising incidence of infectious diseases[82] drug resistance and mutation. Although to kill bacteria is not our aim since bacteria are not cause of disease, but disease lies in person himself (i. e. susceptibility) and bacteria is merely a secondary cause.[83] In the wonderful science of Homoeopathy of defense mechanism is done by minuteness of homeopathic drugs, as Arndt-Shultz law States that "Large doses kill; Moderate dose inhibit; and small doses stimulates", that isa system of treatingdisease by giving extremelysmallamounts of naturalsubstances that, if given in largeramounts to healthypeople, would produce the same effects as the disease[84].

Considering the above arguments, debates, controversies and scientific evidences, the present study has undertaken to find homoeopathy medicines for *in vitro* effective antimicrobial activity against human pathogens. The common and frequently reported cases of human infectious diseases in Bangladesh are diarrhea and related diseases and most of them are caused by enteric bacteria. Every year people of Bangladesh especially infants, elders, and malnourished and immunocompromized group of people have been suffering from diseases caused by enteric human pathogens. Based on preliminary reports, Merc-Cor-6C, Merc-Sol-6C, and Merc-Sol-7C, Pyrogenium-6C, Herper Sulp-6C, Sulfur-6, and Sulfur-7C type homoeopathy medicines are considered and given priority for this investigation. These homoeopathy medicines are applied against common enteric human pathogens, such as *Salmonella typhi*, *Shigella dysenterae*, *Vibrio cholerae*, and *Escherichiacoli*. *Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus* were also applied as test organisms for determining the standard reference organism for further tests.

A brief introduction of the homoeopathy medicines and the test organisms are given below:

1.21. Homoeopathy medicines tested

1. Mercurius Corrosivus-6 (Merc Cor-6)

Synonyms – Mercuric chloride, corrosive sublimate , Mercurius sublimatus

Source- Mineral kingdom

Preparation- It is available from a chemist`s shop. Triturations are prepared from the salt with sugar of milk, from which higher potencies are prepared.
Proved by – Dr. Samuel Hahnemann, Buchner & Massalot.

2. Mercurius Solubilis Hahnemanni-6 (Merc Sol-6)
Synonyms – Merc Solubilis Hahnemanni, Hydrargyrum oxydum nigrum Hahnemanni , Mercury oxide black Hahnemanni, Ammoniated nitrate of mercury, Hydrargyrum, Quick silver, Metallic mercury.
Source- Mineral kingdom
Preparation- Trituration is prepared with sugar of milk. Higher potencies are prepared from 3C of each drug.
Proved by – Dr. Samuel Hahnemann.

3. Pyrogenium: Pyrogenium-6,
Synonyms – Pyrexin, Pyrozen
Source- A nosodes
Preparation- it is product of decomposition of chopped lean beef in water, allowed to stand in the sun for two or three weeks. Dilutions should be made directly according to Burnett, and without glycerine.
Proved by – Dr. Drysdale , Dr. Burnett , Dr.Swan , Dr.Yingling , Dr.Sherbino, Dr. Heath and Dr. H.C.Allen.

4. Hepar Sulfuris Calcareum-(Hepar Sulp-6),

5. Sulfur-6

Synonyms – Sulfur sublimatum , Flores Sulfuris, Sublimed Sulfur, Folwer of Sulfur, Sulfur ,Brimstone –Hindi- Gandhak.
Source- It is an element, occurring in nature as a brittle crystalline solid substance in mines or near volcanoes. It is a compound word consisting of “Sul” meaning salt and “Phur” fire. Hence it means `The salt that burns’. It is called “Gandhak”in Hindi because it has a smell of its own. The medicinal properties of Sulfur were known to Ayurvedic physicians of ancient India who used to prepare and use Makardwaja, a preparation of Sulfur, Mercury and Gold to their patients, Sulfur is available in the market.

Preparation- Trituration of 'Flower of Sulfur' are prepared with sugar of milk and higher potencies are prepared from 3c.A saturated solution of Sulfur in absolute alcohol constitutes the mother tincture.

Proved by – Dr. Sammuel Hahnemann.

1.22. Test enteric organisms

Infectious diseases are very common in our country as well as other developing countries and account for about 50% of all diseases. Drug resistance to human pathogenic bacteria has been reported from every part of the world. Therefore, there is a great need to discover and develop new methods for combating microbial infectious diseases,

1. *Salmonella typhi*:

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motileenterobacteria and flagella which grade in all directions (i.e. peritrichous).*Salmonella* infections are zoonotic and can be transferred between humans and non-human animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis atelic and *Salmonella* typhoid/paratyphoid *Salmonella*, where the latter—because of a special virulence factor and a capsule protein (virulence antigen)—can cause serious illness, such as *Salmonellaenterica* subsp. enterica serovar Typhi. *Salmonella typhi*.is adapted to humans and does not occur in other animals.[85]

Salmonella species are facultative intracellular pathogens that enter cells via macropinosomes.

Most people infected with *Salmonella* develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In most cases, the illness lasts four to seven days, and most people recover without treatment. However, in some cases the diarrhea may be so severe that the patient becomes dangerously dehydrated and must be taken to a hospital. At the hospital, the patient may receive intravenous fluids to treat the dehydration, and may be given medications to provide symptomatic relief, such as fever reduction. In severe cases, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness.[86,87]

2. *Shigella dysenteriae*

Shigellae are Gram-negative, non-motile rods. Shigellae are transmitted by the direct faecal–oral route. As a consequence, food has the potential to be contaminated through the soiled fingers of patients or carriers. The transfer of shigellae by flies breeding on faeces has been established as a very important transmission route during some outbreaks. *Shigella* can be found in surface waters and also within contaminated drinking water. *Shigella* can be classified into four major serological groups. Group A, *Shigella dysenteriae*, Group B, *Shigella flexneri*, Group C, *Shigella boydii*, and Group D, Shigella sonnei, which includes only one serotype. *Shigella sonnei* accounts for most cases of dysenteryin the developed world. Little is known about Shigella and its ability to form and survive in biofilms, which suggests the need for further research. Drinking water treatment that includes disinfection has been shown to be sufficient to remove *Shigella*. However, waterborne outbreaks have generally resulted from inadequate treatment.

3. *Vibrio cholera*e:

Vibrio is a genus of Gram-negative bacteria, possessing a curved-rod shape (comma shape), several species of which can cause foodborne infection, usually associated with eating undercooked seafood. Typically found in salt water, *Vibrio* species are facultative anaerobes that test positive for oxidase and do not form spores. All members of the genus are motile and have polar flagella with sheaths.

Cholera is regarded by many as a disease of great historical importance that marks many of the leaps we have made in the understanding of infectious diseases. Like many diseases of historical significance such as plague, the spread of cholera was also believed to be via bad air or ‘miasma’. It was not until the reports of John Snow’s findings in 1854 that a connection between contaminated drinking water and cholera began to be recognized. John Snow showed that most cholera deaths in a particular region of London were clustered around an area where people acquired water from the same pump on Broad Street. He showed that if an individual cholera victim lived away from the Broad Street vicinity, they did sample this specific pump because they sometimes preferred the taste of the water from there. The removal of the handle of Broad Street pump and the resultant drop in the cases of cholera is heralded by many as the beginning and birthplace of the field of Epidemiology. The identification of *Vibrio cholera* as the etiological agent of cholera was made by Robert Koch in 1894,soon after he proposed the ‘germ theory of infection’or as it is called today, ‘Koch’s

Postulate'. Though clearly an ancient disease, cholera is still common in many regions of the world despite having one of the simplest known treatment regimes: oral rehydration. Moreover, since 2007, the incidence of cholera has gradually increased and the World Health Organisation (WHO) reported 317534 cases and 7543 deaths in 2010 (WHO weekly epidemiological records 2008 and 2011). Since current WHO guidelines no longer require notification of cholera cases, the true burden of the disease is only estimated but is believed to be in the millions every year. For example, not even a single case of cholera has been reported from Bangladesh since 2009, a situation clearly far from the true incidence level. In an attempt to acknowledge the dire global situation relating to cholera, a new resolution has recently been adopted by the WHO for an integrated and comprehensive global approach to the disease (http://www.who.int/cholera/technical/Resolution_CholeraA64_R15-en.pdf). The concern is valid because cholera is a toxin-mediated disease that involves rapid onset of severe watery diarrhea that can lead to the death of a patient within hours if rehydration therapy is not promptly administered. The historical and current impact of cholera on humanity and in generally shaping societies, especially in the developing world, is arguably enormous and this is evident from the mention of the disease in old literature and novels written around it (Sack, et al., 2004). "Asiatic cholera", as it was once called, has now spread globally in the form of epidemics or pandemics and even today it is on the verge of becoming endemic in several countries that generally face hygiene and sanitation problems or are suffering the aftermath of natural disasters, such as Haiti (2010; Barzilay, et al., 2013; Chin, et al., 2011). Several vaccines for protection against cholera are available in the international market but they are not extensively used in low income countries (Lopez, et al., 2008). Therefore the current best approach to cholera control is improvement of hygiene and sanitation where it is most needed, alongside active monitoring of outbreaks in both endemic and epidemic settings. Currently we lack a detailed understanding as to how V. cholera moves around and evolves, although water is clearly a critical factor. In areas where complete and sustained access to clean water is missing, a better understanding of the bacterium and its' general epidemiology is required. Recently, partly provoked by a high profile cholera outbreak in Haiti, as well as ongoing efforts, scientists around the globe have become aware of the paramount importance of continuous and retrospective surveillance using accurate systems such as molecular technologies for tracking and understanding this disease.

V. cholera bacteria live in shallow, salty water on microscopic crustaceans. They can also exist as colonies of biofilms that coat the surface of the water, plants, stones, shells, and

similar items, and they can live among the eggs of midges, which serve as a reservoir for cholera bacteria. Toxic strains of cholera bacteria produce a poison that triggers violent diarrhea in humans.

Only around 1 in 20 cholera infections are severe, and a high percentage of infected people show no symptoms.

If symptoms appear, they will do so between 12 hours and 5 days after exposure. They range from mild or asymptomatic to severe.

They typically include:

- large volumes of explosive watery diarrhea, sometimes called "rice water stools" because it can look like water that has been used to wash rice
- vomiting
- leg cramps

A person with cholera can quickly lose fluids, up to 20 liters a day, so severe dehydration and shock can occur.

Signs of dehydration include:

- loose skin
- sunken eyes
- dry mouth
- decreased secretion, for example, less sweating
- fast heart beat
- low blood pressure
- dizziness or lightheadedness
- rapid weight loss

Shock can lead to collapse of the circulatory system. It is a life-threatening condition and a medical emergency.

Cholera bacteria enter the body through the mouth, often in food or water that has been contaminated with human waste, due to poor sanitation and hygiene.

They can also enter by eating seafood that is raw or not completely cooked, in particular shellfish native to estuary environments, such as oysters or crabs.

Poorly cleaned vegetables irrigated by contaminated water sources are another common source of infection.

4. *Escherichia coli* 0157:H7 [93]

Escherichia coli are normal inhabitants of the human large intestine. Most strainsare harmless, but some strains acquire bacteriophage or plasmid DNA-encoding enterotoxins or invasion factors and become pathogenic. These virulent strains are responsible for diarrheal infections worldwide, as well as neonatal meningitis,septicemia, and urinary tract infections (UTIs).

E coli are gram-negative bacilli of the family Enterobacteriaceae. They are facultative anaerobes and nonsporulating. *E coli* strains with the K1 capsular polysaccharide antigencause approximately 40% of cases of septicemia and 80% of cases of meningitis. Different strains of *E coli* are associated with a number of distinctive diarrhealillnesses (Table). Among these are the enterotoxigenic *E coli* (ETEC), entero-invasive *E coli* (EIEC), and Shiga toxin–producing *E coli* (STEC). Of the STEC,E coli O157:H7 is the prototypic strain. Each class of *E coli* has distinct somatic (O)and flagellar (H) antigens and specific virulence characteristics.

Diarrheogenic *E coli* strains are worldwide in distribution. The route of infectionis fecal-oral, predominantly via contaminated water and food. STEC, especially *E. coli* O157:H7, is shed in feces of cattle, sheep, deer, andother ruminants. Human infection is acquired via contaminated food or water or via direct contact with an infectedperson. Outbreaks have been linked to ground beef, exposureto animals in public settlings (petting zoos), contaminatedapple cider, and contamination of water in recreational areas.The incubation period for most Ecolistrains is 10 hours to6 days. For *E coli* O157:H7, the incubation period is usually 3to 4 days.

The source of *E coli* and other Gram-negative bacterial pathogens in neonatal infections is often through the maternal genital tract. Hospital acquisition of gram-negative organisms through person-to-person transmission from nursery personnel or environmental sites can occur. The incubation period is variable with time of onset of infection ranging from birth to several weeks after birth

Objectives

- Identification of Homoeopathy medicine which shows sensitivity against microorganisms

- Detection of sensitivity against microorganisms by using Homoeopathy medicines.

- Determination of MIC and MBC of active drugs against enteric human pathogens.

- Determination of bio-potency of Homoeopathy medicines in comparison to azithromycin.

CHAPTER: 02

MATERIALS AND METHODS

2. 1. Materials

2.1.1. Selecting homoeopathy medicines for antibacterial activity

Some homoeopathy medicines are selected based on preliminary antibacterial properties against human ailments. These are:

1. Mercurius corrosivus-6 (Fig.2.1.a),
2. Mercurius solubilis-6 (Fig.2.1.b),
3. Sulfur -6 (Fig.2.1.c),
4. Heparsulfuris calcareum-6 (Fig.2.1.d),
5. Pyrogenium-6 (Fig.2.1.e),
6. Dynamized Merc-sol-7 and
7. Dynamized sulfur-7

The purchased homoeopathy medicines are shown in Fig 2.1

a **b** **c** **d** **e**

Fig.2.1 Image of homoeopathy medicines used for antibacterial activity against enteric human pathogens. (a) Mercurius corrosivus-6, (b) Mercurius solubilis-6, (c) Sulfur-6, (d) Herparsulfuris calcareum-6, (e) Pyrogenium-6

2.1.2. Test organisms

Human enteric pathogens:-

1. *Escherichia coli* O157:H7 (ATCC 12079).
2. *Shigella dysenterae* , (ICDDR'B)
3. *Salmonella typhi* (ICDDR'B),
4. *Vibrio cholera* ATCC 6395, (ICDDR'B) and

Human non-enteric pathogens:-

5. *Bacillus cereus*,
6. *Staphylococcus aureus*, ATCC 25923, (ICDDR'B)
7. *Micrococcus luteus* (ICDDR'B)

Human non-enteric pathogens were selected to find reference strain which is sensitive to all homoeopathy medicines studied.

2.1.3. Media used

1. Tryptic soy agar (TSA) (NISSUI, Japan)— for periodic transfer of test organisms
2. Tryptic soy broth (TSB) (NISSUI, Japan)— for preparation of seed culture
3. Müeller-Hinton Agar (MHA) Medium (Becton, Dickinsm and Company, France)- for antibacterial sensitivity test,
4. Müeller-Hinton Broth(MHB)(Becton, Dickinsm and Company, France) for inoculum preparation.

2.2. Methods

2.2.1.Handling of laboratory apparatus and glassware

All glassware was washed with mild detergents, rinsed 4-5 times in tap water and finally rinsed twice in distilled water before use and dried in air. When needed, glassware likePetri plates were heat sterilized at 180°C for Ih in hot air oven (Binder ED23, Germany)before use. Micropipette tips, glass pipette and Eppendorf tubes were sterilized by autoclaving at 121°C for 15 min at 15 psi (Hirayama, Model HA-300M, Japan).

2.2.2.Preservation and Maintenance of the test organisms

The rest organisms were sub cultured on TSA plates for pure colony by streak plate

method(Fig.2.2). Aliquot from an individual colony of each organism was transferred aseptically onto TSA slant in tube(Fig.2.3) and incubated at 37^{o}C for 24 hours, and then the slant was kept at refrigerator temperature, which was used as working culture and the working culture used to transfer onto new slants every 15 days of interval to keep culture live. A part of the discrete colony was transferred by a sterile inoculating loop inTSB and was incubated at 37°C (approximately 16 hours culture). Then 0.80 ml of culture wastransferred to sterile cryogenic vial (Fig.2.4) containing 0.20 ml of sterile glycerol and mixed well and kept at -20°C for long term preservation of the test organisms.

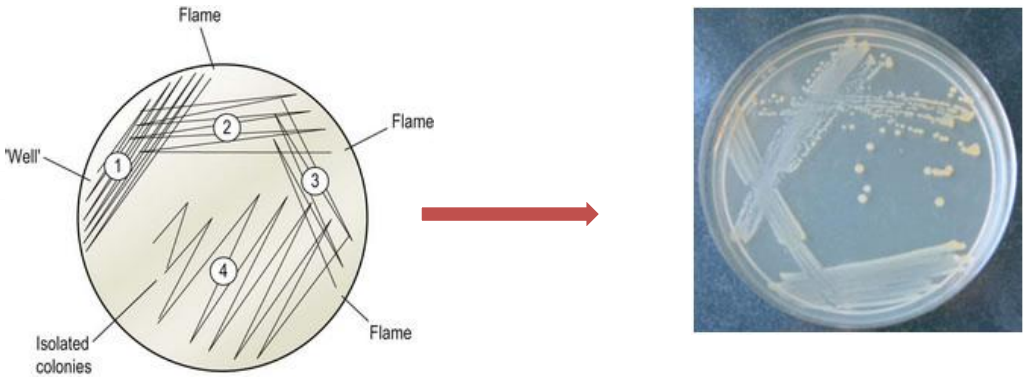
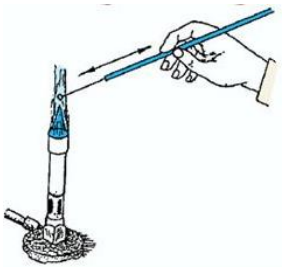
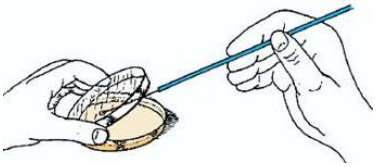


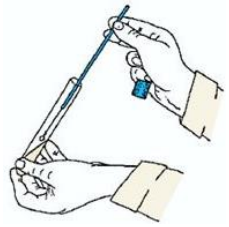
Fig.2.2. Detection of pure culture (Representative image) of the test organisms by streaking method



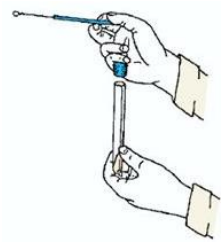
1. Inoculated loop is heated until it is red-hot



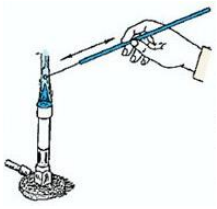
2. With free hand, the lid of the plate raisedjust to access a colony to pick up a loopful of organisms



3. After flaming the mouth of a sterile slant, it's surface wasstreaked



4. The mouth of the tube was flamedand re-caped the tube



5. The inoculating loop was flamedand returned it to receptacle

Fig.2.3. Inoculating an agar slant from an agar plate

Fig 2.4. Sterile Cryovials

2.2.3. Screening of selective homeopathic medicines for antibacterial activity against human enteric pathogens

The antibacterial activity of the homoeopathy medicines was carried out and assayed by the disc diffusion methods described by Bauer *et al*.(1966).[94] This method was elaborately described by Saha*et al*. (2017).[95]

2.2.3.1. Impregnation of disc with selected homeopathic medicines

Discs (6mm) were impregnated with 50µl and 100µl separately of each homoeopathy medicine and were dried at 40^{o}C for half an hour in a hot air oven (Barnstead Labline, USA) and were stored at 4^{o}C until use. Negative control was prepared only with 95% ethanol.

2.2.3.2. Preparation of inocula

One loopful of inoculum of each test organism from cryogenic vial was transferred into 9 ml of sterile Tryptose Soy Broth (TSB) and grown at 37^{o}C for 24 hours. One loopful of the TSB culture was then streaked into the TSA plate and grown at 37^{o}C for 24 hours. The inocula of the test organisms were prepared by transferring 3 or 4 colonies of the cultures on TSA into 9 ml of sterile MHB and incubated at 37^{o}C for 5 to 6 hours, if necessary 12 to 18 hours was considered.[95] The MHB culture was compared with McFarland standards (10^{8}CFU/ml).[96]

2.2.3..3. Inoculation of previously prepared MHA plates

After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The dried surface of a MHA plate was inoculated by streaking the swab over the the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^{o}C each time.

2.2.3..4. Application of homoeopathy medicine impregnated discs onto inoculated agar plates

The homoeopathy medicine impregnated discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. For each plate five discs were placed, of which three discs were impregnated with homoeopathy medicine, one disc was impregnated with 95% ethanol (negative control), and one disc was azithromycin, a positive control (15 µg/disc). The plates were inverted and placed in an incubator at 37^{o}C for 24 hours. All these procedures were applied for all homoeopathy medicines for determination of their antibacterial activity against human enteric and non-enteric pathogens.

2.2.3..5. Evaluation of antibacterial activity of homoeopathy medicines

Antibacterial activity of the homoeopathy medicines against test organisms employed were evaluated by measuring the zones of inhibition in mm (including the 6mm disc) with digital slide calipers near the agar surface and the results were recorded. The end point was taken as complete inhibition of growth of the test organisms as determined by the naked eye. Each homoeopathy medicine was tested in triplicates and assay of this experiment was repeated thrice.

2.2.4. Determination of MIC and MBC of the active homoeopathy medicines by Broth Dilution

1. Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below:

(a) Fourteen (14) sterile tubes were placed in a rack and were labeled each 1 to 14

13th test tube was labeled as Positive control and last one (14th) was labeled as Negative control.

(b) 0.9 ml of Mueller Hinton broth was taken in each test tube and 0.1 ml of test organism culture was added in it.

(c)1 ml of test drug was added to test tube No. 1.

(d) With a sterile micropipette and tips, after adequate mixture 1 ml was transferred from tube No. 1 to tube No. 2.

(e) After a through mixing, 1 ml was transferred with a separate micro pipette from tube No 2 to tube No 3.

(f) This procedure was repeated through the next-to-next up to the tube No. 12. except tube No 13 and 14 (using fresh pipette for each dilution). From tube No. 12, 1ml was removed and discarded. 13th tube served as Positive control and received bacterial culture. The last tube(14th) received test drug and served as a Negative control.

2. The tubes were incubated at 37◦C for 24 hours.
3. The tubes were examined for growth and were determined the MIC of tested medicine, which is bacteriostatic for the test organism. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-).

For determination of MBC, the concentration which was bactericidal, was then found by sub cultured the contents of selective tubes into a series of Mueller Hinton agar.

2.2.5. Determination of Bio-potency of the active homoeopathy medicines[97]

Purpose

1. For determining the potency of the homoeopathy medicines active against human enteric pathogens
2. For determining the pharmacokinetic of the homoeopathy drugs.
3. For monitoring and controlling antimicrobial chomotherapy.

Principle:

Many antibiotics are used as chemotherapeutic agents which are ideally,toxic only for pathogenic microorganisms and are harmless to thehost. But some are too highly toxic for use in case of chemotherapeutic use, and/or some against which pathogens have become resistant. In this case homeopathic medicines may be the harmless alternatives. An antibiotic assay of antibiotics includes certain methods such as microbiological assays, radio enzymatic assays, high performance or high pressure liquid chromatography(HPLC), immunoassays etc.

Microbiological assay can be readily accepted not only as an essential part of the analytical and production control but also an invaluable technique for investigational work. There are three main methods of microbiological assay by which the potencyof sample and standard solution may be compared:

I . Dilution methods

2. Diffusion methods

3. Turbidimetric, titrimetric and gravimetric methods

The disadvantages of dilution, turbidimetric, titrimetric and gravimetric methods arewell recognized. These are as follow -

I .A low throughput of samples only is possible.

2. Comparatively low degrees of precision unless increased replication andclose spacing of dilution levels are employed.

3. Extensive incubator and bench space is required

4. Difficulties in preparing an assay design to avoid or reduce sources of error.

5. Critical statistical analysis may be most complicated

6. Results in some cases may be not be available for to 5 days.

Considering these disadvantages, it is found that diffusion methods are preferable for assaying potency of antimicrobials.

The agar diffusion method, the mostly used method of microbiological assay is followed in this experiment. In agar diffusion assays, the response of a growing population of microorganisms to the antimicrobial agent is measured. So, this method is used to determine the potency of the antimicrobials. Potency is a term used to express the strength of a chemical and bio-potency is a process used to express the strength of antimicrobials. Antimicrobials, originated from microorganisms, plants, animals, and minerals also undergo chemical formulations and thereby need to be evaluated in terms of potency for clinical use as well as for market value while assaying the potency of an antimicrobial, proper calibration is essential to express the result in terms of absolute units. Thus a pure sample of the drug or a sample of known potency is essential for the preparation of calibrator solutions.

The agar diffusion assay may be one dimensional, two-dimensional or three dimensional. One dimensional assay is less used today. Rather two or three dimensional assay is the commonest form of microbiological assay. This experiment employs the three dimensional agar diffusion assays. In this system samples are applied in reservoir (well) to a thin layer of agar seeded with indicator organism(s).The drug diffuses into the medium. After incubation a zone of growth inhibition forms (a circle around the reservoir). The diameter of the zone is related to the concentration of drugs in the reservoir.

The edge of alone is formed when the minimum concentration of antimicrobials which will inhibit growth of organism(s) on plate (critical concentration) reaches for the first time (when the population density is too great for the antimicrobials to inhibit). The position or the zone edge is thus determined by

1. The initial population density

2. Growth rate of the organism

3. The rate of diffusion of antibiotic

4. Agar thickness.

A balanced design is one in which all controllable variables have been accommodated and such design is 'Latin square design'. A Latin square arrangement is usually acceptable for pharmacokinetic or clinical assays. In situations where the concentration range of tests will lie in a narrow range and where high precision is sought, a Latin Square Design with tests and calibrators at 2 or 3 levels of concentration may be used. As a result this assay ensures maximum precision. Different types of Latin Square Designs are known. In this experiment, the potency of homoeopathy medicines such as merc-sol-6 and sulfur-6 are determined by three dimensional agar diffusion assay using 4x4(2+2) Latin Square Design.

The 4x4(2+2) Latin Square Design is used for the assay of are sample and one standard at two levels each and allows four zones for each level in a tree Latin Square design where each treatment appears once in every row and in every column. The plate size is7'/4x 7'/4 inches.

(1) <u>Preparation of bacterial suspension:</u>

Bacterial *(E. coli* O157:H7*, Shi. dysentare, Sal. typhi, and V. cholerae)* suspension was prepared in such away that it wasallowed25 %light transmission at 580 nm, which was spectrophotometrically measured.

(2)Preparation of standard solution

a) Sample to be taken

$$X = \frac{10 \times 100}{\text{Standard potency}}$$

Where, x is the actual amount of standard sample taken. Potency of supplied standard Azithromycin was 95%

So,

$$X = \frac{10 \times 100}{95}$$

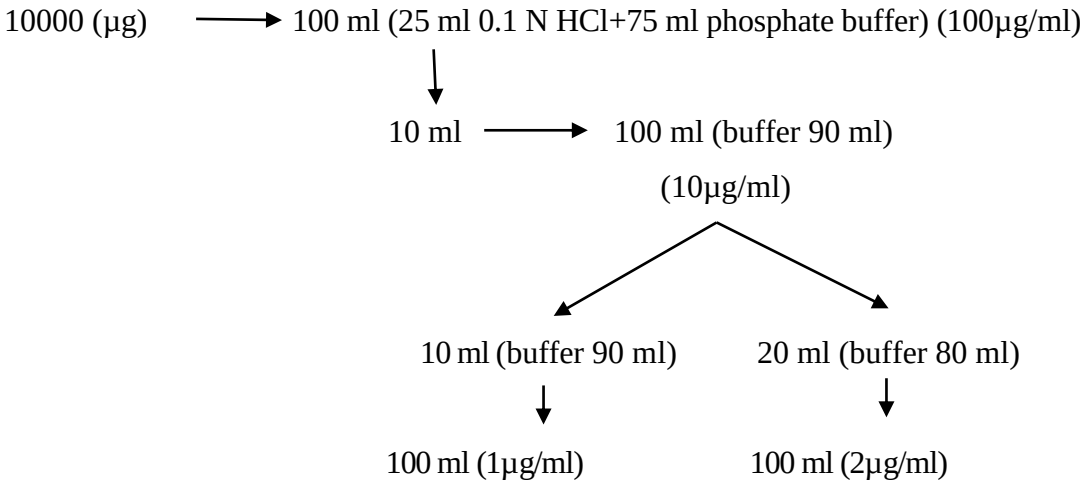
= 10.53 mg

Then the measured sample was taken the conical flask. Then 25 ml of 0.1 N HCl and 75 ml of phosphate bufferwere added in the flask. After addition of HCl and buffer concentration become 100 µg/ml by 100 base dilution process, it was diluted

to2µg/ml (higher concentration and 1 µg/ml (lower concentration).**In** each dilutaion of azithromycin buffer was used.

Dilution:

X



(4)After finishing all these preparations, 0.75 ml bacterial suspension was added to150 ml molten nutrient agar media (50°C) in conical flask and mixed by swirling toavoid bubble.

(5)The inoculated molten agar medium (50°C) was poured into Petri plate aseptically and the plate was allowed to stand for a few minutes for solidification.

(6)For further solidification the nutrient agar plate was kept at refrigerator (4°C) for a few minutes.

(7)Then using a sterile borer, 16 wells were made designed in such a way that every well apart from the same distance.

(8) 100 microlitre of each of the standard and the test solution were dropped in wells in the following sequence:

Std (H) ⟶ Std (L) ⟶ Sam (H) ⟶ Sam (L)

(9)Then the plate was kept undisturbed for about half an hour and then incubated at37°C for 24 hours.

(10)After incubation a zone of inhibition was measured and assay work sheet was prepared.

2.2.6 Calculation:

Potency =Antilog (D/B x I) x F x H[98]

D= (T1+ T2) – (S1+ S2)

B= (T1- T2) + (S1- S2)

I=Log ratio of dilution used

F= Factor of dilution used (Highest dilution)

H= Highest dilution used

S1= Zone of Inhibition for highest concentration of reference standard

S2=Zone of Inhibition for Lowest concentration of reference standard

T1= Zone of Inhibition for Highest concentration of test solution

T2=Zone of Inhibition for Lowest concentration of test solution

2.3. Determination of dynamization of merc-sol-6 and sulfur-6

One-third of one hundred grains sugar of milk is put in a glazed porcelain mortar, the bottom dulled previously by rubbing it with fine, moist sand. UPON THIS POWDER is put one grain of the powdered drug to be triturated (one drop of quicksilver, petroleum, etc.). The sugar of milk used for dynamization must be of that special pure quality that is crystallized on strings and comes to us in the shape of long bars. For a moment the medicine and powder are mixed with a porcelain spatula and triturated rather strongly, six to seven minutes, with the pestle rubbed dull, then the mass is scraped from the bottom of the mortar and from the pestle for three to four minutes, in order to make it homogeneous. This is followed by triturating it in the same way 6-7 minutes without adding anything more and again scraping 3-4 minutes from what adhered to the mortar and pestle. The second third of the sugar of milk is now added, mixed with the spatula and again triturated 6-7 minutes, followed by the scraping for 3-4 minutes and trituration without further addition for 6-7 minutes. The last third of sugar of milk is then added, mixed with the spatula and triturated as before 6-7 minutes with most

careful scraping together. The powder thus prepared is put in a vial, well corked, protected from direct sunlight to which the name of the substance and the designation of the first product marked 100 is given. In order to raise this product to 10000, one grain of the powdered 100 is mixed with the third part of 100 grains of powdered sugar of milk and then proceed as before, but every third must be carefully triturated twice thoroughly each time for 6-7 minutes and scraped together 3-4 minutes before the second and last third of sugar of milk is added. After each third, the same procedure is taken. When all is finished, the powder is put in a well corked vial and labeled 10000. If now, one grain of this last powder is taken in the same way, the 1/1,000,000, I. E., (1), each grain containing 1/1,000,000 the original substance. Accordingly, such a trituration of the three degrees requires six times six to seven minutes for triturating and six times 3-4 minutes for scraping, thus ONE HOUR for every degree. After one hour such trituration of the first degree, each grain will contain 1/000; of the second 1/10,0000; and in the third 1/1,000,000 of the drug used (see Note1). Mortar, pestle and spatula must be cleaned well before they are used for another medicine. Washed first with warm water and dried, both mortar and pestle, as well as spatula are then put in a kettle of boiling water for half an hour. Precaution might be used to SUCH AN EXTENT as to put these utensils on a coal fire exposed to a glowing heat.[104]

Based on the process described above 1ml Sulfur 6 was mixed with 99 ml Rectified Spirit, and 100 succussion (the action or process of shaking or the condition of being shaken especially with violence) were performed on an elastic material. After 100 consecutive succession, Sulfur 6 and Merc-sol-6 were dynamized into Sulfur-7 and Merc-sol-7.

CHAPTER: 03

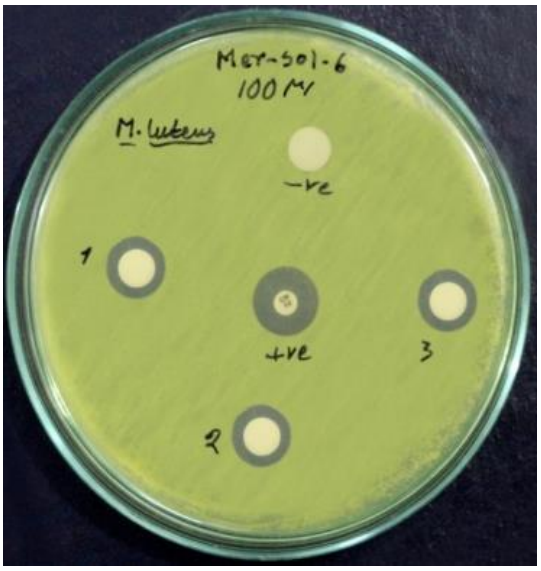
RESULTS

3.1 Determination of reference strains for antibacterial activity of homoeopathy medicines against human pathogens

For preparation of reference bacterial strain susceptible to the test homoeopathy medicines *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus luteus* were tested with homoeopathy medicines by disc diffusion method (Bouer, et al.). The results are shown in Fig. 3.1 and Table 3.1.

(a) Merc-Sol-6 against *Staphylococcus*

(b) Merc sol-6 against *B. cereus*



(c)Marc-Sol-6 against *M.luteus*

Fig.3.1. In vitro antibacterial activity of Merc-sol-6 against (a) *S. aureus*, (b) *B. cereus* and (c) *M. luteus* on MHA plates.

Table 3.1a. Antibacterial activity of 5 common homoeopathy medicines against *Bacillus cereus, Staphylococcus aureus* and *Micrococcus luteus*

Test organisms	Homoeopathy medicines tested (Zones of inhibition in mm)						Negative control	Positive control
	Merc-Cor-6(µl/disc)		Merc.-Sol-6 (µl/disc)		Sulfur-6 (µl/disc)		Ethanol (%)	AZM
	50	100	50	100	50	100	95	15µg/disc
B. cereus	0	0	14.0 ±0.24	19.0 ±0.30	10.0 ±0.30	22.0 ±0.18	0	19.0±0.20
S. aureus	0	0	10.0 ±0.30	12.0 ±0.25	13.0 ±0.22	18.0 ±0.15	0	18.0±0.15
M. luteus	18.0 ±0.19	23.02 ±0.12	19.67 ±0.31	24.67 ±0.34	19.67 ±0.32	24.13 ± 0.20	0	22.8±0.36

Table 3.1b. Antibacterial activity of 5 common homoeopathy medicines against *Bacillus cereus, Staphylococcus aureus* and *Micrococcus luteus*

Test organism	Homoeopathy medicines tested (Zones of inhibition in mm)				Negative control	Positive control
	Heper sulfur-6 (µl/disc)		Pyrogen-6 (µl/disc)		Ethanol	(AZM)
	50	100	50	100	95%	15 µg/disc)
B. cereus	0	0	0	0	0	19.0±0.20
S. aureus	0	0	0	0	0	18.0±0.15
M. luteus	12.33±0.28	20.24±0.14	16.30±0.14	19.05±0.08	0	22.8±0.36

All five homoeopathy medicines showed antibacterial activity against *M. luteus*, of which merc.-sol-6 and Sulfur-6 showed the highest activity than other three medicines.

From the experimental findings (Table 3.1a,b) it was found that *Micrococcus luteus* was sensitive to all five homoeopathy medicines studied, for which this bacterium was considered as reference organism for further study.

3.2 Screening of antibacterial activity of homoeopathy medicines against enteric human pathogens

The five homoeopathy medicines viz Merc-Cor-6, Merc.-Sol-6, Sulfur.-6, Heper sulfur.-6, and pyrogenium-6 were applied against four enteric pathogens such as *E. coli*, *Shigella* sp., *Salmonella typhi*, and *V. cholerae* by disc diffusion method.[94].

From the experimental findings (Table 3.2 andFigs.3 2a,b and Figs.3.3.a,b) it was found that all test organisms were resistant to Merc-cor-6, Heper Sulfur 6 and Pyrogenium-6, but sensitive to marc-sol;.-6 and Sulfur-6.

Table 3.2. Antibacterial activity of 5 homoeopathy medicines against common 4 enteric pathogens

Test organisms	Homoeopathy medicines tested (Zones of inhibition in mm)				Negative control	Positive control
	Merc.-Sol-6 (µl/disc)		Sulfur-6 (µl/disc)		Ethanol (%)	Azithromycin (AZM)
	50	100	50	100	95	(15 µg/disc)
E. coli 0157:H7	13.1±0.20	20.09±0.21	12.67±0.31	20.13±0.3	0	18.34±0.15
Shigella dysenteriae	10.5±0.33	15.45±0.15	10.33±0.20	22.33±0.18	0	16.58±0.16
S. typhi	15.3±0.18	19.13±0.30	14.33±0.28	21.67±0.35	0	16.24±0.18
V. cholerae	14.33±0.24	19.46±0.20	9.13±0.20	18.05±0.08	0	17.33±0.18
M. luteus Ref. organism	19.67±0.31	24.67±0.34	19.67±0.32	24.13±0.20	0	23.00±0.17

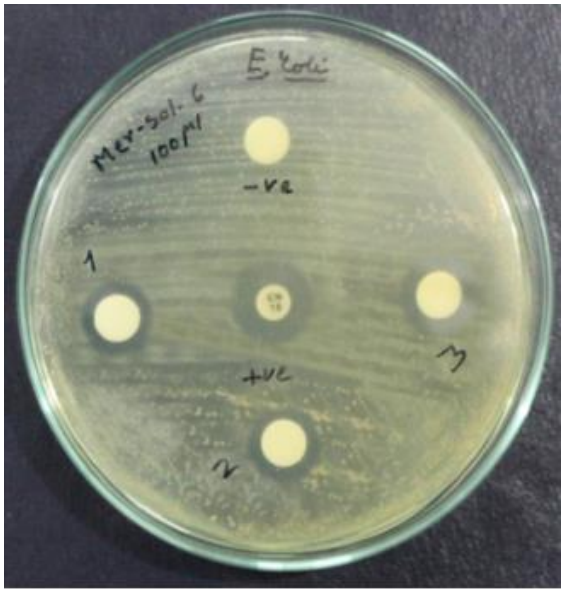
MercCcor-6, Herper-Sulfur-6 and Pyrogenium-6 showed no zones of inhibition.

Merc-sol-6 showed the highest activity against *S. typhi* and the lowest against *S. dysenteriae,* on the other hand, Sulfur-6 showed the highest activity against *Shi.* dysenterae and lowest against *V. cholerae*. Both categories of the medicines showed higher activity against *M. luteus*. Comparatively, the Sulfur-6 showed a little bit of higher activity than that of Merc-Sol-6. 95% ethanol showed no activity, because at 96% concentration ethanol is frequently evaporated from the disc. 100 µl/disc of merc-sol-6 and Sulfur-6 showed significant higher activity than that of azithromycin (AZM).

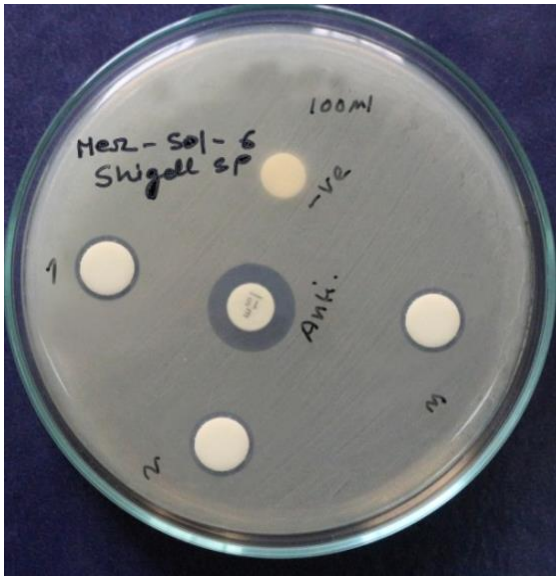
Merc-Sol-6 and Sulfur-6 were potentitized to Merc-Sol-7 and Sulfur-7, respectively and their antimicrobial properties against *E. coli 0157:H7*, *Shigella dysenterae*, *Salmonella typhi* and *Vibrio cholera* were determined and results are shown in Table 3.3. From the experimental findings it was noticed that activity of both medicines at potency 7, was diminished at insignificant level. No further works were done with these two medicines at potencies 7, because of time constraint.

Table 3.3. Antibacterial activity of Merc-Sol-7 and Sulfur-7 against 4 enteric pathogens

Test organisms	Homoeopathy medicines tested (Zones of inhibition in mm)				Negative control	Positive control
	Merc.-Sol-7 (µl/disc)		Sulfur.-7 (µl/disc)		Ethanol (%)	Azithromycin (AZM)
	50	100	50	100	95	(15 µg/disc)
E. coli 0157:H7	9.8±0.33	11.17±0.71	9.17±0.41	11.5±0.70	0	18.34±0.15
Shigella dyseneteriae	8.5±.33	10.25±.06	8.33±.02	10.33±.08	0	16.58±0.16
S. typhi	8.33±0.41	10.33±0.47	8.33±.08	9.37±.06	0	16.24±0.18
V. cholerae	9.33±.12	10.21±.2	8.23±.2	11.05±.08	0	17.33±0.18

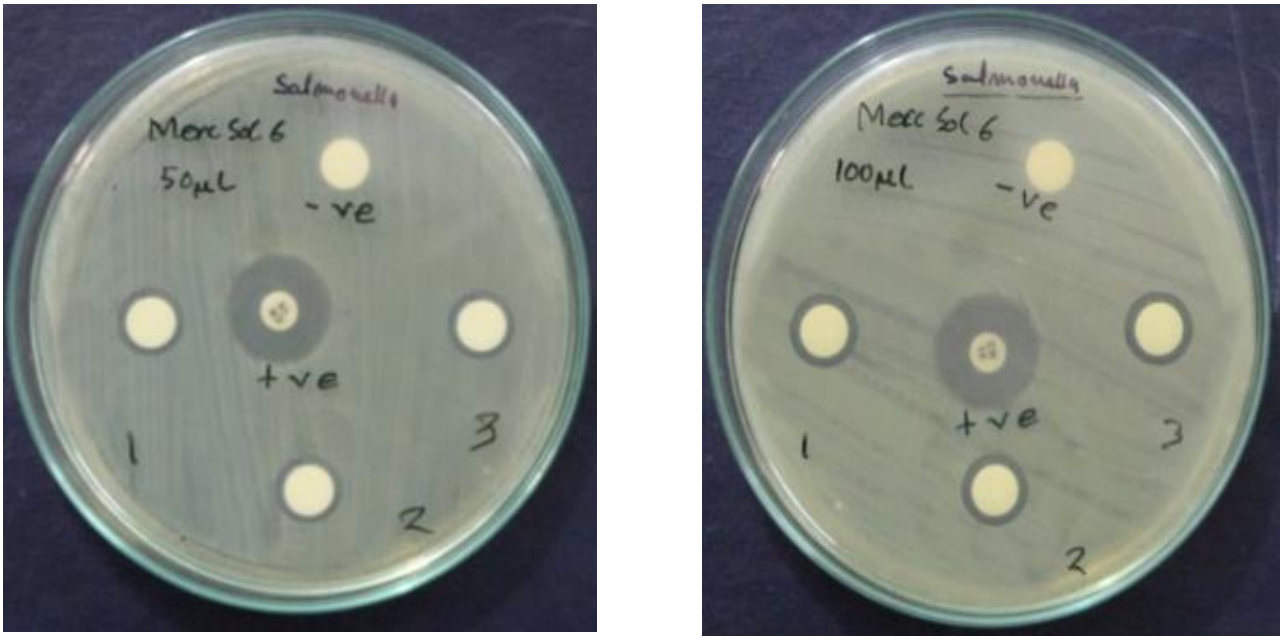


(a) Merc-Sol-6 against *E. coli 0157:H7*

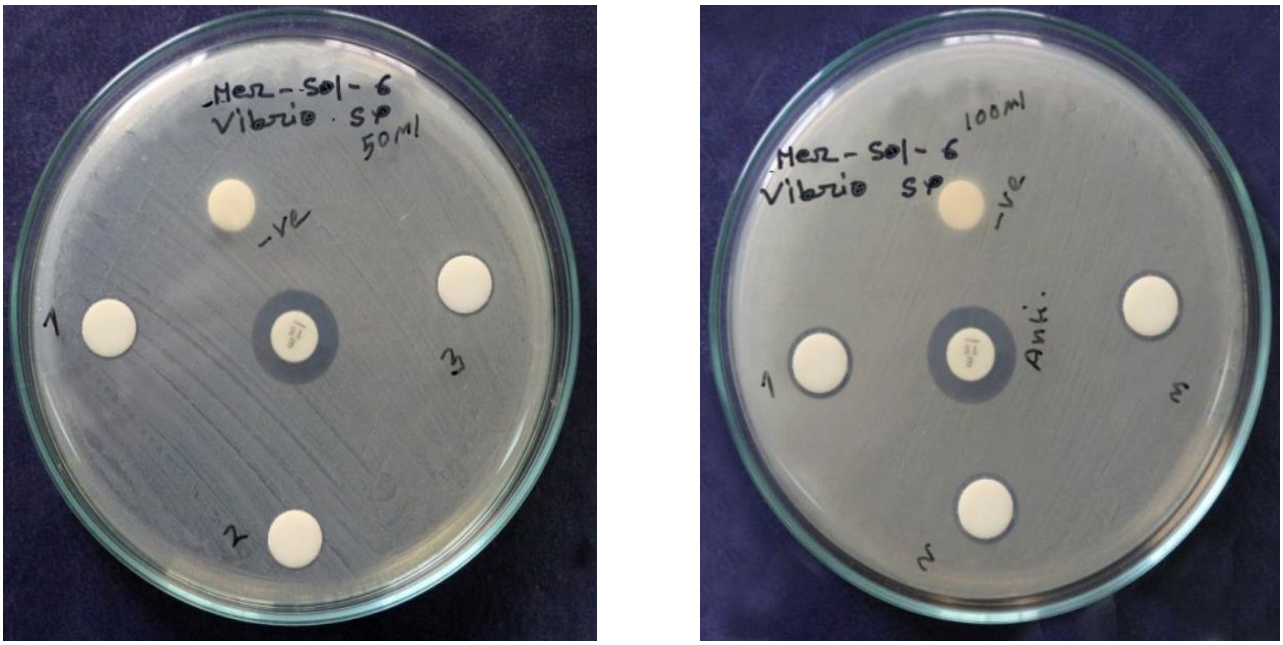


(b)Merc-sol-6 against *S. dysenterae*

Fig.3.2a. Photograph of *in vitro* antibacterial activity of Merc-Sol-6 against (a) *E. coli* (with 50µl/disc and 100 µl/disc), and (b) *S. dysenterae*(with 50µl/disc and 100 µl/disc),

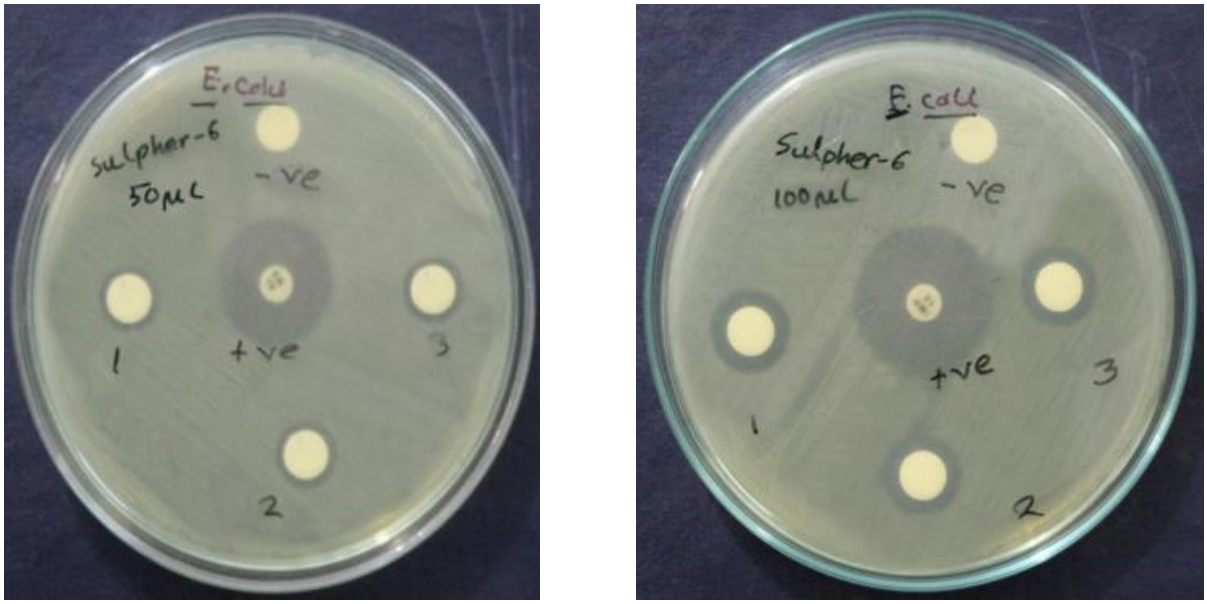


(c) Merc-Sol-6 against *Salmonella typhi*

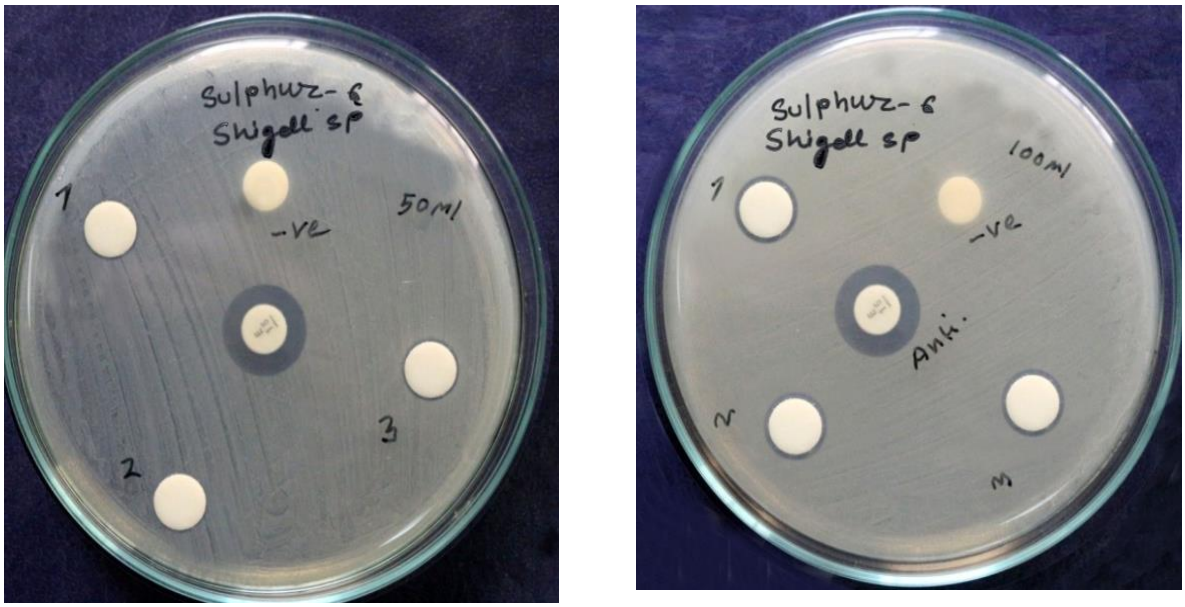


(d) Merc-sol-6 against *V. cholerae*

Fig.3.2b. Photograph of *in vitro* antibacterial activity of Metc-sol-6 against (c) *Salmonella typhi*(with 50µl/disc and 100 µl/disc), (d) *V. cholerae*(with 50µl/disc and 100 µl/disc) on MHA plates.

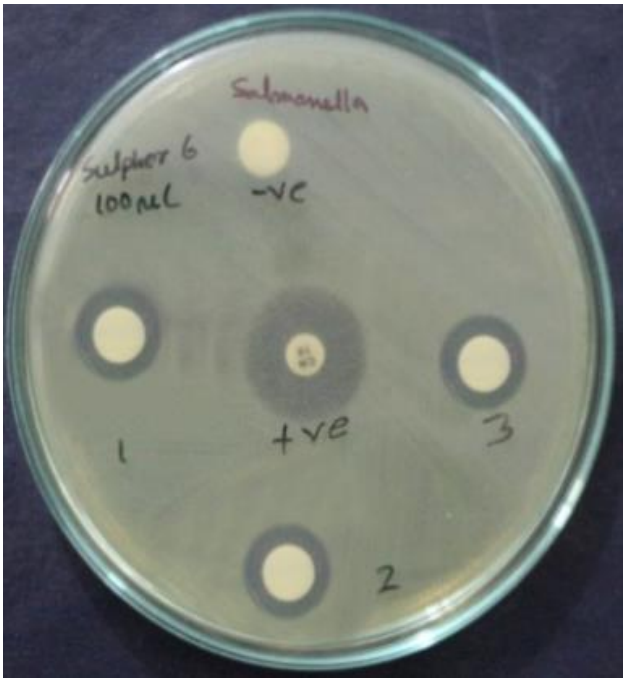


(a) Sulfur-6 against *E. coli 0157:H7*

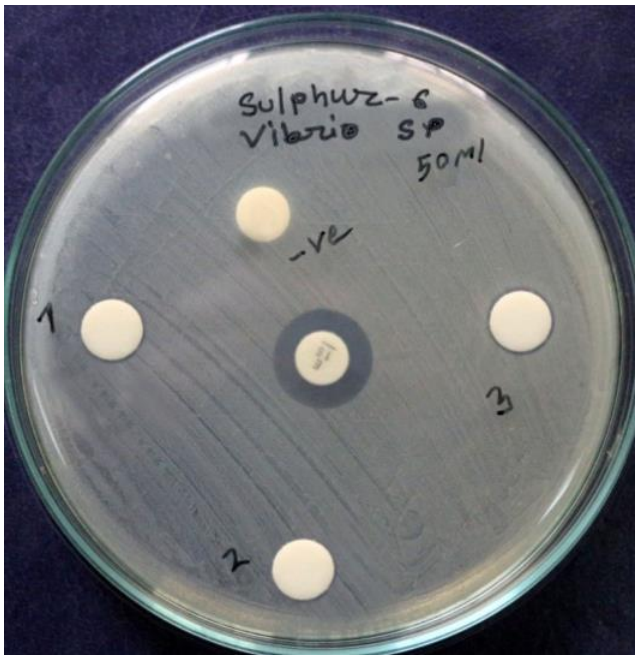


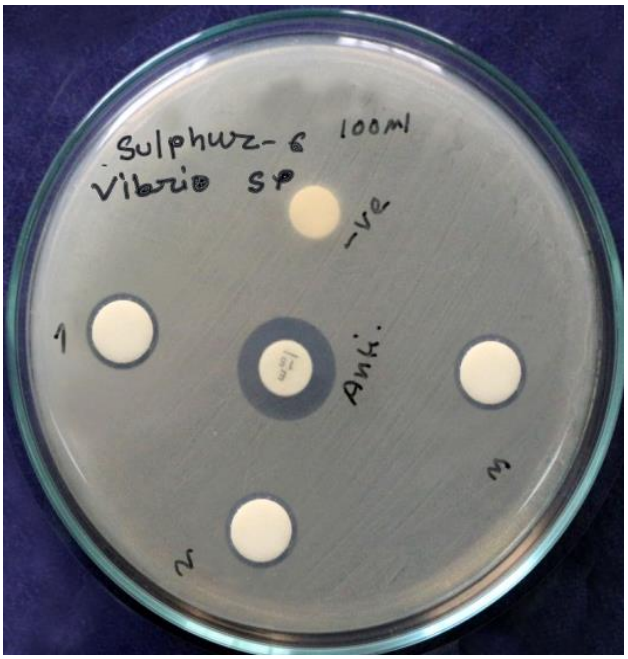
(b) Sulfur-6 against *Shigella dysenterae*

Fig.3.3a. Photograph of *in vitro* antibacterial activity of Sulfur-6 against (a) *E. coli 0157:H7* (with 50µl/disc and 100 µl/disc), (b) *Shigella dysenterae*(with 50µl/disc and 100 µl/disc),



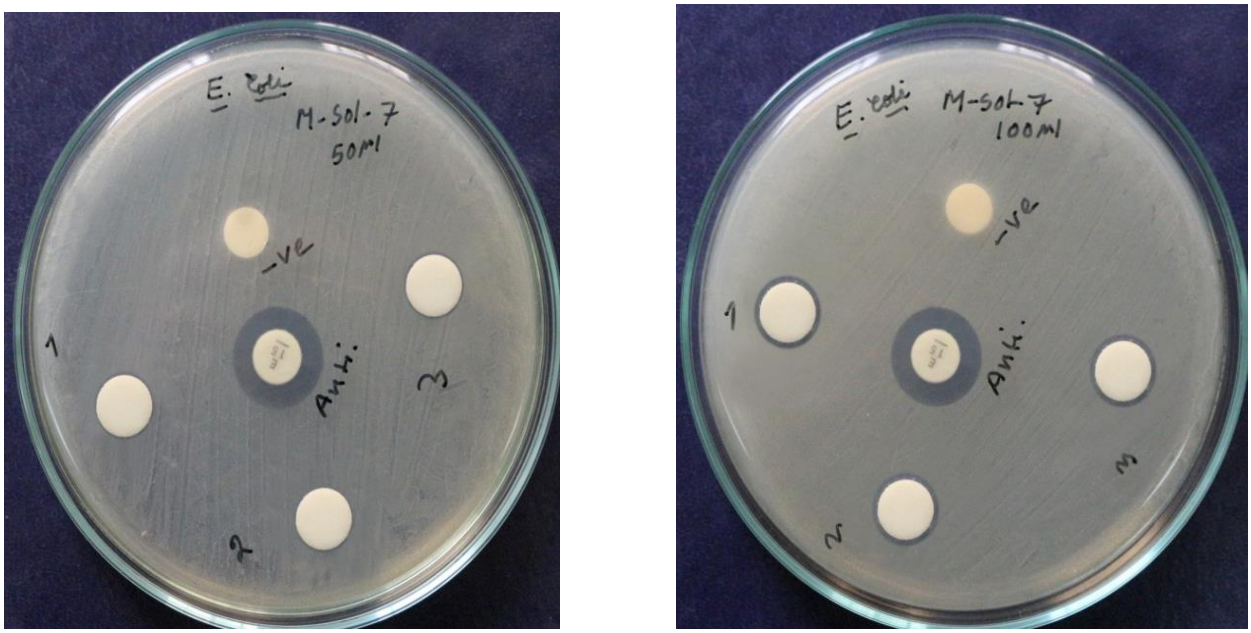
(c) Sulfur-6 against *Salmonella typhi*





(d) Sulfur-6 against *V. cholerae*

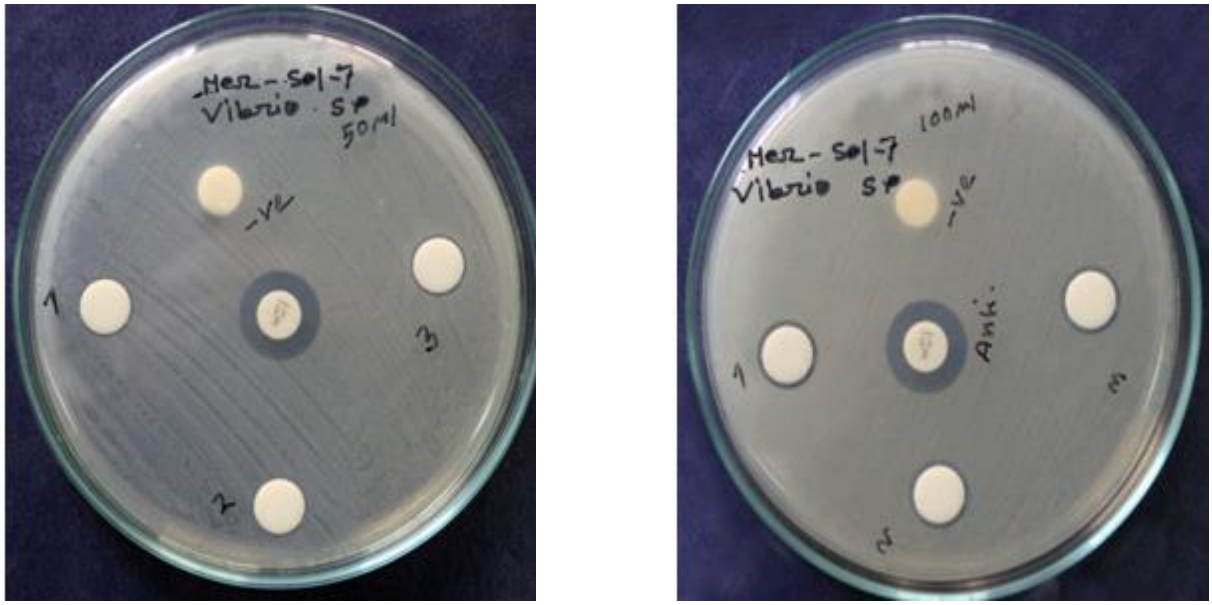
Fig.3.3b. Photograph of *in vitro* antibacterial activity of Sulfur-6 against (c) *Salmonella typhi* (with 50µl/disc and 100 µl/disc), (d) *V. cholera* (with 50µl/disc and 100 µl/disc) on MHA plates.



(a) Merc-sol-7 against *E coli 0157:H7*

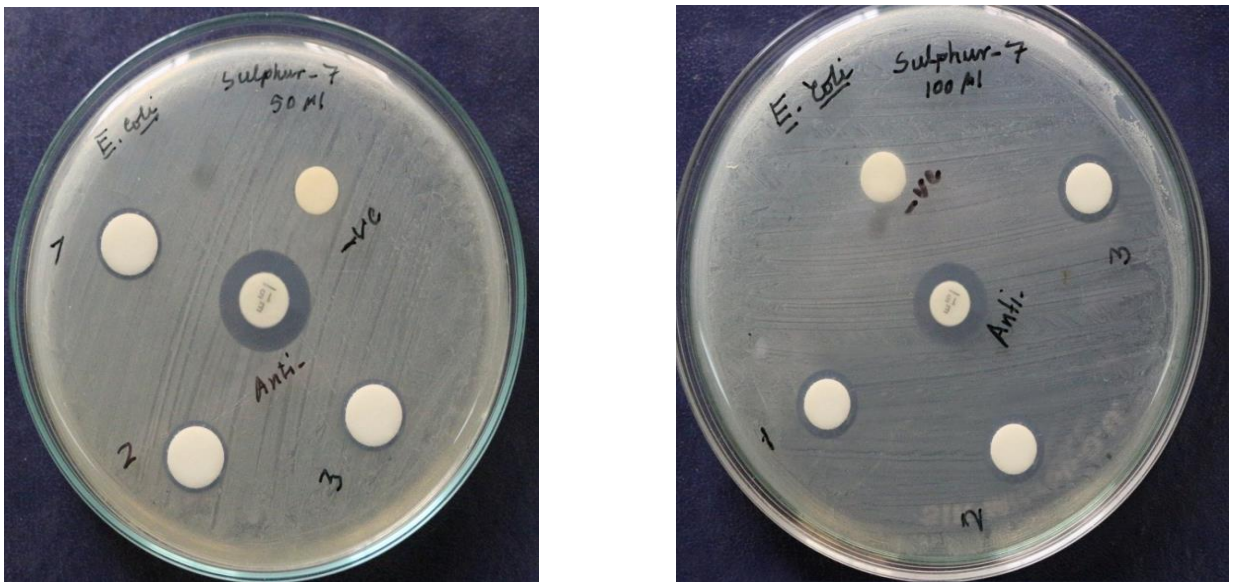
(b) Merc-Sol-7 against *Shigella dysenterae*

Fig.3.4a. Photograph of *in vitro* antibacterial activity of Merc Sol -7 against (a) *E. coli* (with 50µl/disc and 100 µl/disc), (b) *Shi .dysenterae*(with 50µl/disc and 100 µl/disc),



(c) Merc-sol-7 against *Vibrio cholerae*

Fig.3.4b. Photograph of *in vitro* antibacterial activity of Merc Sol -7 against *Vibrio cholerae* (with 50µl/disc and 100 µl/disc),



(a) Sulfur-7 against *E coli*

Fig.3.5a. Photograph of *in vitro* antibacterial activity of Sulfur-7 against (a) *E. coli* (with 50µl/disc and 100 µl/disc)

(b) Sulfur-7 against *Shigella dysenteriae*

(c) Sulfur-7 against *Vibrio cholerae*

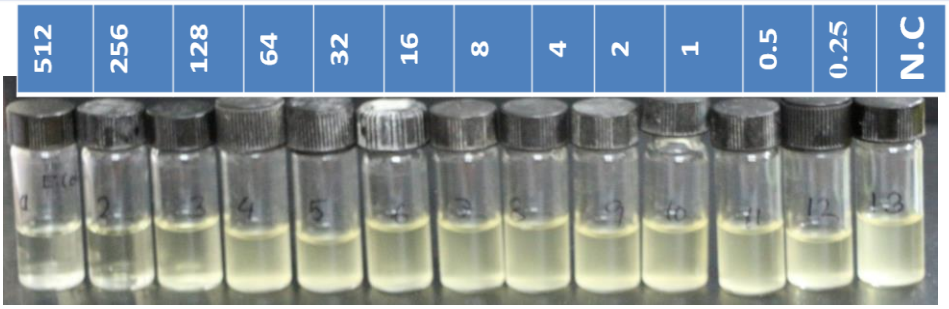
Fig.3.5b. Photograph of *in vitro* antibacterial activity of Sulfur-7 against (b) *Shigella dysenterae*(with 50µl/disc and 100 µl/disc) (c) *Vibrio cholerae*(with 50µl/disc and 100 µl/disc)

3.3 **Determination of MIC and MBC of Merc-Sol-6 and Sulfur-6**

MIC and MBC of Merc-Sol-6 and Sulfur-6 were carried out against their susceptible pathogens such as *E. coli*, *Shigella dysenterae*, *Salmonella typhi*, and *V. cholerae*. Results are shown in the following tables with their figures (Tables 3.4-3.11; Figs. 3.4-3.11).

Table 3.4. MIC and MBC of Merc-sol-6 against *Escherichia coli*

Test tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Merc-sol-6-6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024 µg	512 µg	256 µg	128µg	64 µg	32 µg	16 µg	8 µg	4 µg	2 µg	1 µg	0.5 µg	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	-	-	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	-	-	+	+	+	+	+	+	+	+	+	+	-
MIC- 64µl, MBC- 128µl														



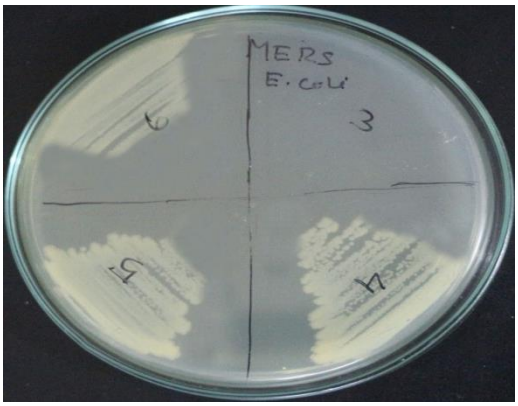


Fig. 3.4.MIC and MBC of Merc-sol-6 against *Escherichia coli 0157 :H7*

Table 3.5. MIC and MBC of Merc-sol-6 against *Shiaella dysenterae*

Test tube no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Merc-Sol -6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	+	+	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	+	+	+	+	+	+	+	+	+	+	+	+	-
MIC- 256µg, MBC- 512µg														

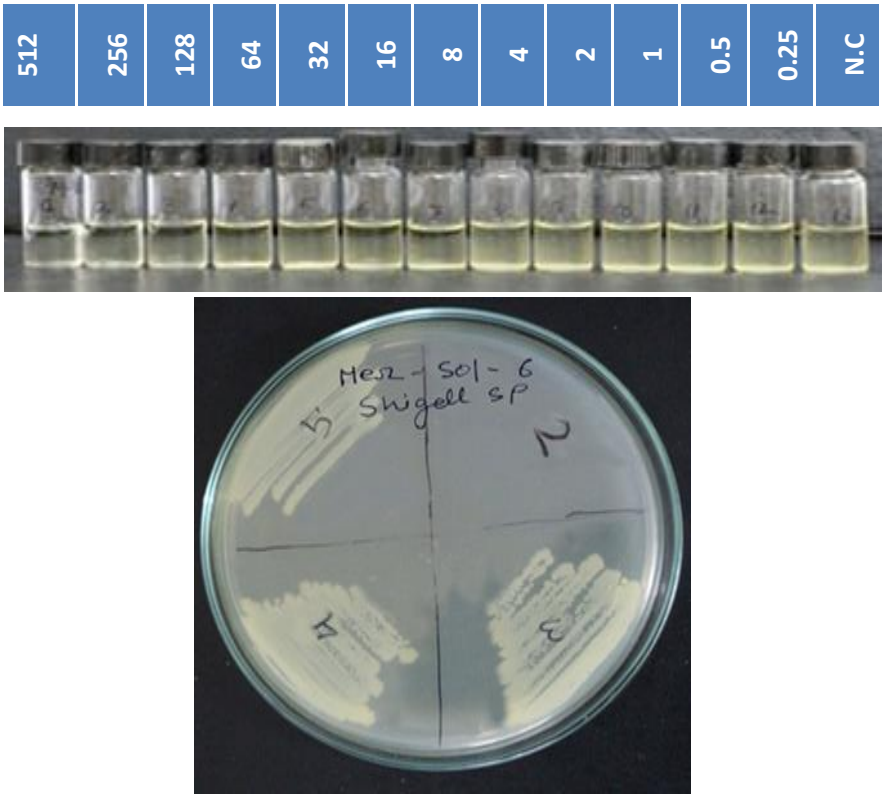


Fig. 3.5.MIC and MBC of Merc-Sol-6 against *Shigella dysenterae*

Table 3. 6. MIC and MBC of Merc - Sol-6 against *Salmonella typhi*

Test tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Merc-sol-6-6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024 µg	512 µg	256 µg	128µg	64 µg	32 µg	16 µg	8 µg	4 µg	2 µg	1 µg	0.5 µg	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	-	-	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	-	-	+	+	+	+	+	+	+	+	+	+	-
MIC- 64µl, MBC- 128µl														

Fig.3.6. MIC and MBC of Merc - Sol-6 against *Salmonella typhi*

Table 3.7.MIC and MBC of Merc-Sol-6 against *Vibrio cholerae*

Test tube no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Merc-sol-6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	+	+	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	+	+	+	+	+	+	+	+	+	+	+	+	-
MIC- 256µg, MBC- 512µg														

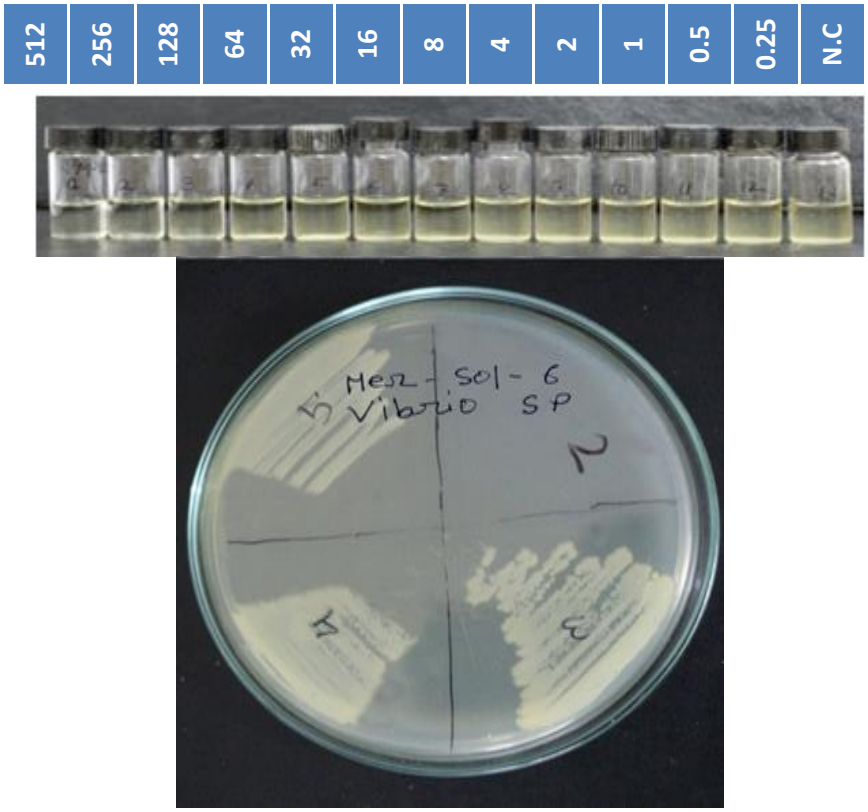
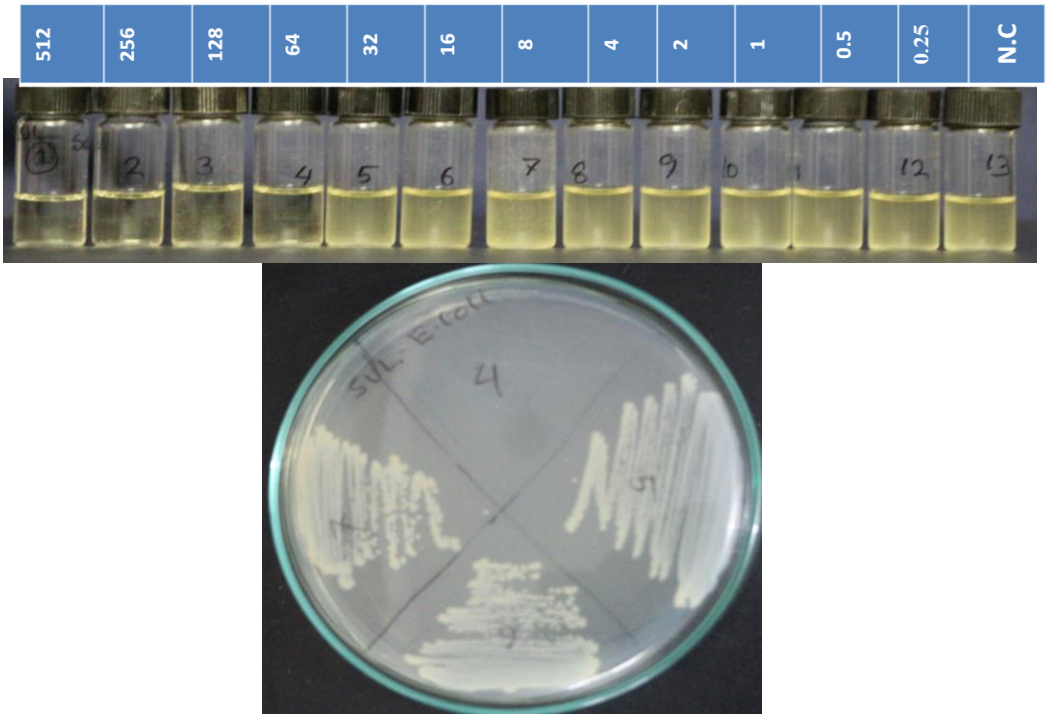


Fig. 3.7.MIC and MBC of Merc-Sol-6 against *Vibrio cholerae*

Table 3.8. MIC and MBC of Sulfur-6 against *Escherichia coli*

Test tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Sulfur -6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	-	-	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	-	-	+	+	+	+	+	+	+	+	+	+	-
MIC- 64µl, MBC- 128µl														



3.8. MIC and MBC of Sulfur-6 against *Escherichia coli*

Table 3.9. MIC and MBC of Sulfur-6 against *Shigella dysentarae*

Test tube no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Sulfur -6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	+	+	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	+	+	+	+	+	+	+	+	+	+	+	+	-
MIC- 256µg, MBC- 512µg														

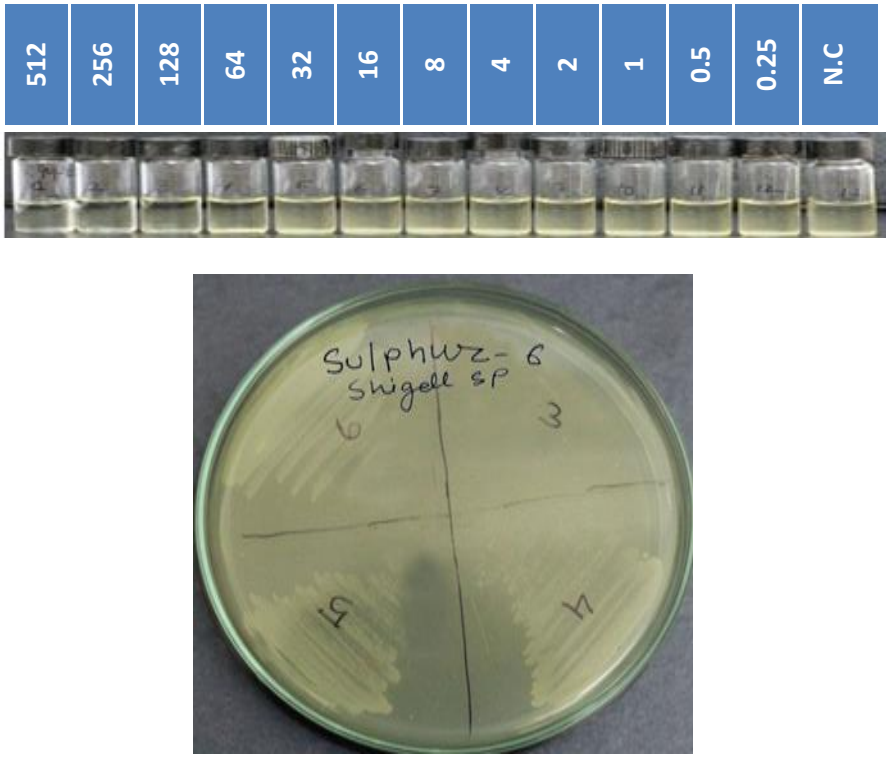


Fig.3.9. MIC and MBC of Sulfur-6 against *Shigella dysenterae*

Table 3.10. MIC and MBC of Sulfur-6 against *Salmonella typhi*

Test tube No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Sulphur -6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024 µll	512 µl	256 µl	128µl	64 µl	32 µl	16 µl	8 µl	4 µl	2 µl	1 µl	0.5 µl	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Broth dilution	-	-	-	-	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	-	-	-	+	+	+	+	+	+	+	+	+	-
MIC- 64µl, MBC- 128µl														

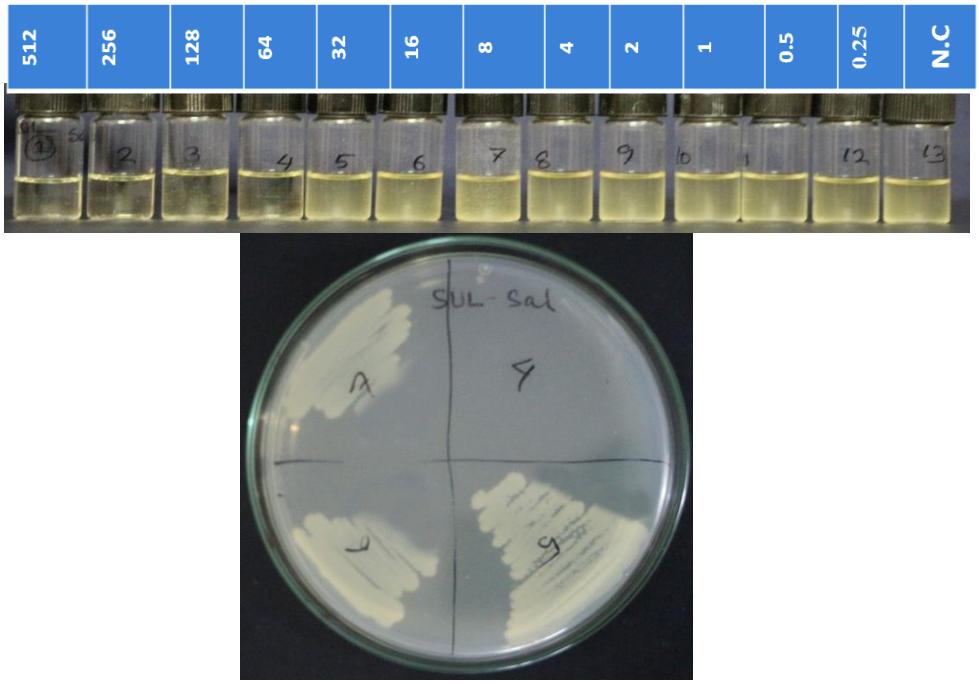


Fig.3.10. MIC and MBC of Sulfur-6 against *Salmonella typhi*

Table 3.11. MIC and MBC of Sulfur-6 against *Vibrio cholerae*

Test tube no	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Muller Hinton Broth (ml)	0	1	1	1	1	1	1	1	1	1	1	1	1	1
Sulfur -6(1024µl)	2	1	1	1	1	1	1	1	1	1	1	1	1	0
Initial antibiotic concentration(µl/ml)	1024	512	256	128	64	32	16	8	4	2	1	0.5	P.C	N.C
Bacterial suspension	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Final volume	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Final concentration (µl/ml)	512	256	128	64	32	16	8	4	2	1	0.5	0.25	-	-
Broth dilution	-	-	-	+	+	+	+	+	+	+	+	+	+	-
Growth on plate	-	-	+	+	+	+	+	+	+	+	+	+	+	-
MIC- 128µg, MBC- 256µg														

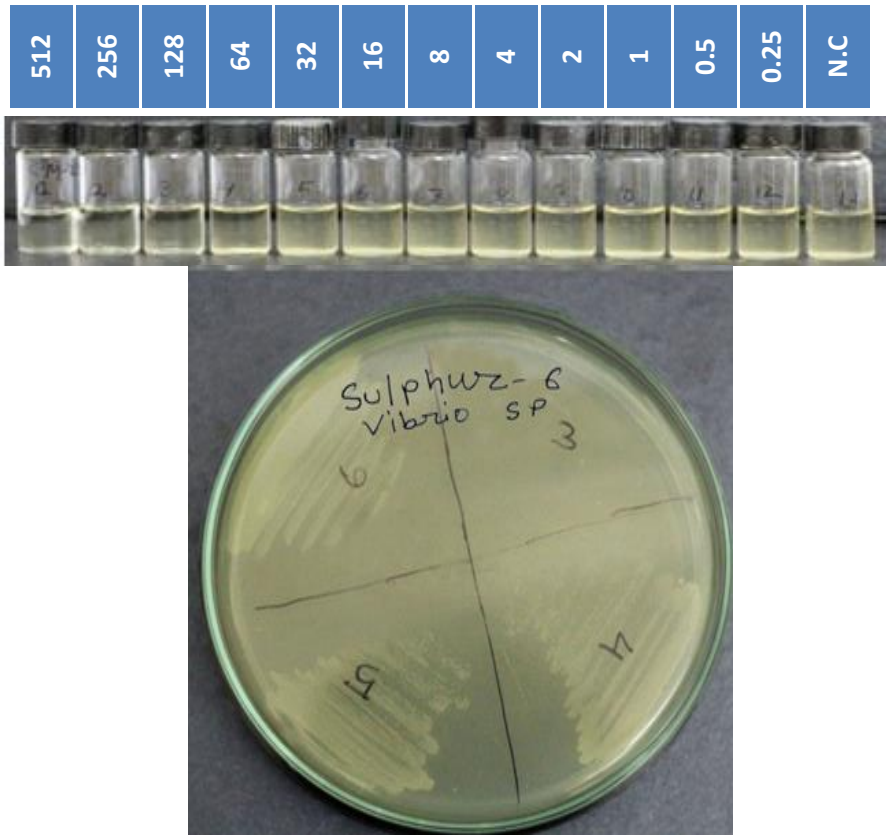


Fig.3.11. MIC and MBC of sulfur-6 against *Vibrio cholerae*

Table 3.12. MIC and MBC of Sulfur-6 and Merc-sol-6 against enteric pathogens

	Merc-sol-6		Sulpher-6	
Test Organism	**MIC in µl/ml**	**MBC in µl/ml**	**MIC in µl/ml**	**MBC in µl/ml**
Escherichia coli	128	256	64	128
Salmonella typhi	64	128	64	128
Shigella dysentriae	256	512	256	512
Vibrio cholerae	256	512	128	256

Some suggestions had been coming from the M. Phil seminar held in the Department of Microbiology, University of Dhaka to carry on antibacterial activity of dynamized merc-sol-6 and sulfur-6 to Merc-Sol-7 and Sulfur-7 at the eleven time of the M. Phil research.The experiments were carried according to the suggestions and results are shown in Table 3.12. MIC, MBC, and bio-potency tests of Merc-Sol-7 and Sulfur-7 were not possible to carry out due to time constrains.

Table 3.13. Antibacterial activity of Merc-Sol-7 and Sulfur-7 against common 4 enteric pathogens

Test parameters	Antibacterial activity (zones of inhibition in mm)					
Test materials	Merc-sol-7		Sulfur-7		Ethanol	AZM
Concentrations / Test organisms	50µl	100µl	50µl	100µl	95%	15µg/disc
E. coli 0157:H7	9.4±.1	11.04±.01	9.37±.01	11.04±.4	0	18.34±.15
S. dysenterae	8.5±.33	10.25±.06	8.33±.02	10.33±.08	0	16.58±.16
S. typhi	8.23±.12	10.13±.3	8.33±.08	9.37±.06	0	16.24±.18
Vibrio cholerae	9.33±.12	10.21±.2	8.23±.2	11.05±.08	0	17.1±.63
M. luteus	9.27±.11	10.36±.02	9.67±.02	10.13±.03	0	22.78±.36

3.4 Biopotency of Merc-Sol-6 and Sulfur-6 in comparison to azithromycin

Biopotency of Merc-Sol-6 and Sulfur- 6 were determined comparing to the antibacterial activity of Azithromycin using agar well diffusion assay. The results are shown in table 3.12 to 3.21 along with calculation.

Table 3.14.Biopotency of Merc-sol-6 against *M. luteus*

	Merc-sol-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
M. luteus	17.2	17.1	20.5	20.6	20.9	20.8	*25.1*	25.2
	17.3	17.4	20.6	20.5	20.7	20.6	*25.3*	25.3
	17.2	17.2	20.5	20.6	20.6	20.7	*25.4*	25.3
	17.2	17.3	20.6	20.5	20.9	20.8	25.1	25.3
sum	T2	68.95	T1	82.2	S2	83	S1	101

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	82.2	68.95	151.15	13.25
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	101	83	184	18

D=(T1+ T2) – (S1+ S2)=-32.85
B=(T1+ T2) + (S1+ S2)=31.25
D/B= -1.0512
I=Log ratio of dilution used=0.301
Antilog (D/B x I)= 0.4831
F= Factor of dilution used (Highest dilution)=**4**
H= Highest dilution used= **10**
Potency =Antilog (D/B x I) x F x H = 19.324

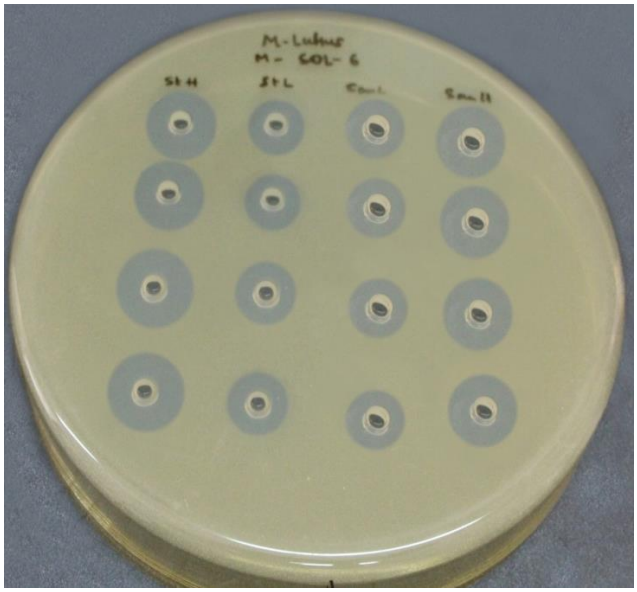


Fig-3.12 Biopotency of Merc-sol-6 against M. luteus

Table 3.14.Biopotency of Sulfur-6 against *M. luteus*

	Sulfur-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
M. luteus	17.2	17.1	20.7	20.5	22	21	*25*	25
	17.3	17.2	20.6	20.5	20	20.4	*25*	25.1
	17.2	17.1	20.7	20.6	20	20.1	*25*	25.1
	17.2	17.1	20.7	20.6	20	20	25	25.2
sum	T2	68.7	T1	82.45	S2	81.75	S1	100.2

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	82.2	68.95	151.15	13.75
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	101	83	181.95	18.4

D=(T1+ T2) – (S1+ S2)=-30.8

B=(T1+ T2) + (S1+ S2)=32.15

D/B= -0.96

I=Log ratio of dilution used=0.301

Antilog (D/B x I)= 0.514

F= Factor of dilution used (Highest dilution)= 4

H= Highest dilution used= 10

Potency =Antilog (D/B x I) x F x H = 20.56

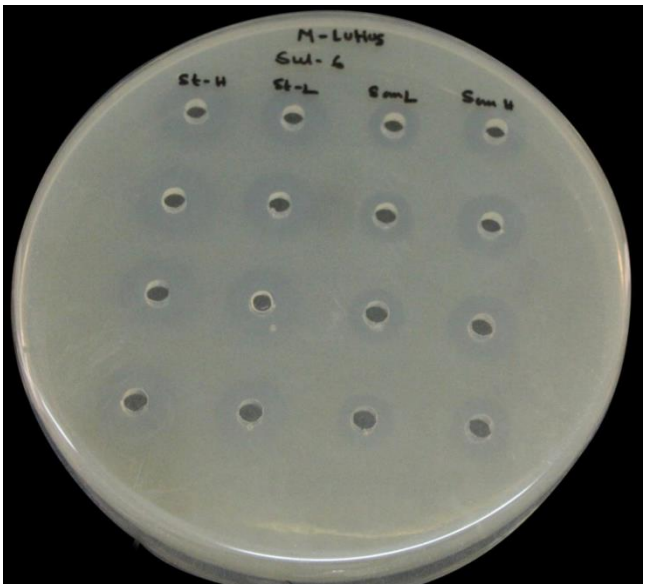


Fig 3.13: Biopotency of Sulfur-6 against *M. luteus*

Table 3.15.Biopotency of Merc-sol-6 against *E. coli 0157:H7*

	Merc-sol-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
E. coli	15.6	15.4	19.8	19.6	19.6	19.5	*24.3*	24.5
	15.5	15.3	19.7	19.8	19.5	19.7	*24.4*	24.5
	15.6	15.4	19.8	19.6	19.6	19.6	*24.3*	24.4
	15.6	15.5	19.8	19.7	19.5	19.7	25.5	24.6
sum	T2	61.95	T1	78.9	S2	78.35	S1	98.25

Merc-sol-6 78.9 61.95 140.85 16.95
Azithrimycin **S1** **S2** **S1+S2** **S1-S2**
98.25 78.35 176.6 19.9

D=(T1+ T2) – (S1+ S2)=-35.75
B=(T1+ T2) + (S1+ S2)=36.85
D/B= -0.97
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.5105
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 20.42

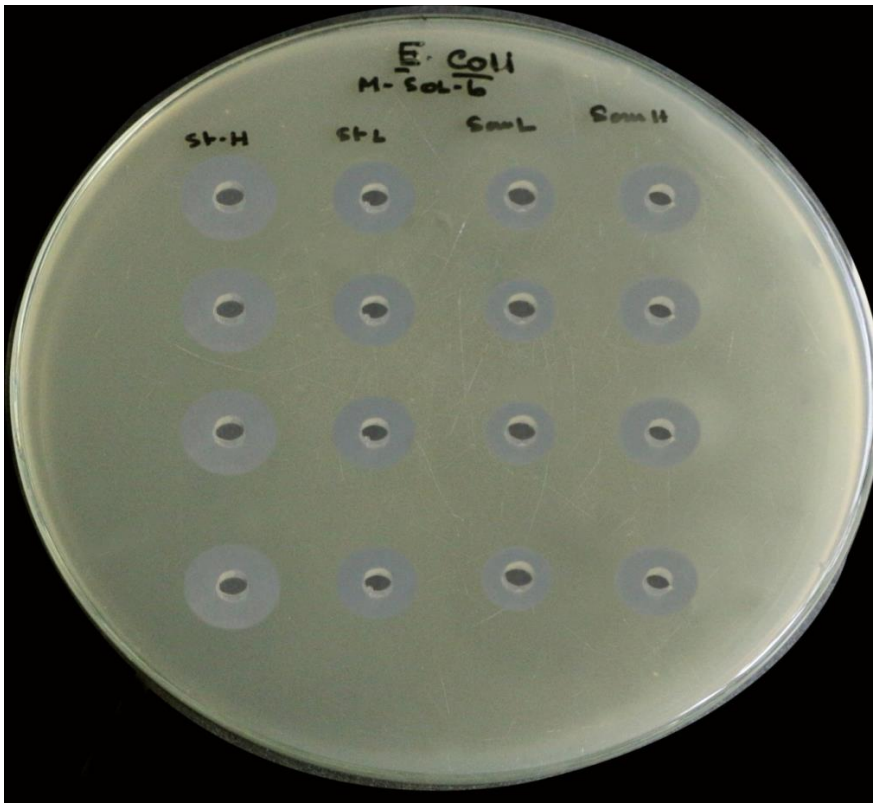


Fig 3.14 - Biopotency of Merc-sol-6 against *E. coli 0157: H7*

Table 3.16.Biopotency of Sulfur-6 against *E. coli 0157:H7*

	Sulfur-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
E. coli	15.6	15.5	19.8	19.8	15.6	15.5	*19.8*	19.8
	15.5	15.6	19.8	19.7	15.5	15.6	*19.8*	19.7
	15.4	15.3	19.7	19.6	15.4	15.3	*19.5*	19.6
	15.2	15.1	19.5	19.6	15.2	15.1	19.5	19.6
sum	T2	61.6	T1	78.75	S2	61.6	S1	78.65

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	78.75	61.95	140.35	17.15
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	98.25	78.35	140.25	17.05

D=(T1+ T2) – (S1+ S2)=-0.1
B=(T1+ T2) + (S1+ S2)=34.2
D/B= -0.002
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.998
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 39.92

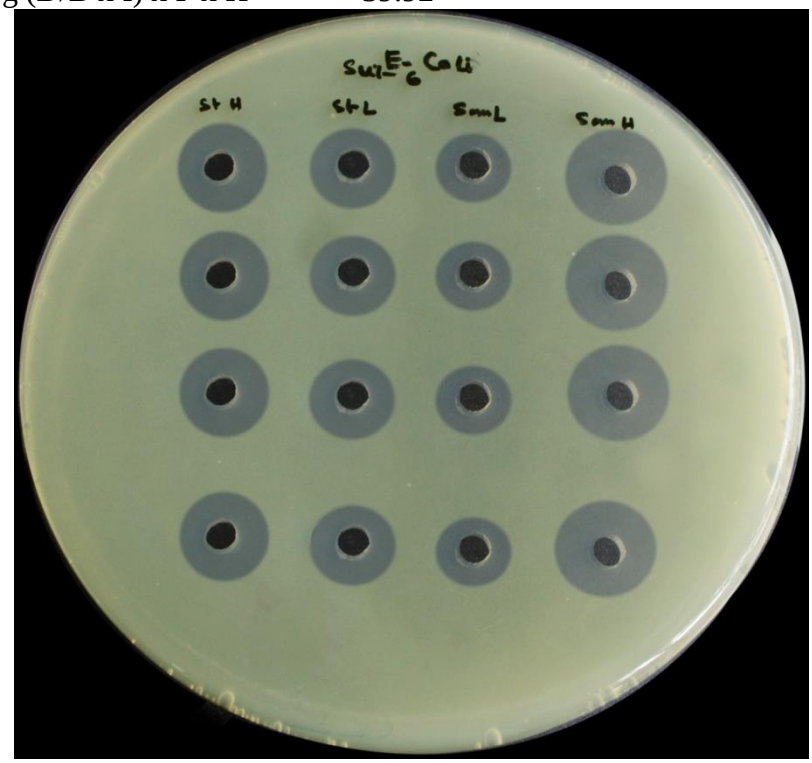


Fig 3.15 - Biopotency of Sulfur-6against *E. coli 0157: H7*

Table 3.17.Biopotency of Merc-sol-6 against S.*typhi*

	Merc-sol-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
S. typhi	17.1	17.2	20.2	20.1	19.5	19.4	*23.1*	23
	17.3	17.2	20.3	20.2	19.6	19.5	*23.5*	23.4
	17.4	17.3	20.4	20.3	19.5	19.4	*23.5*	23.5
	17.1	17.2	20.2	20.1	19.5	19.6	23.4	23.6
sum	T2	68.9	T1	80.9	S2	78	S1	93.5

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	80.9	68.9	149.8 12	
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	93.5	78	171.5	15.5

D=(T1+ T2) – (S1+ S2)=-21.7
B=(T1+ T2) + (S1+ S2)=27.5
D/B= -0.789
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.579
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 23.16

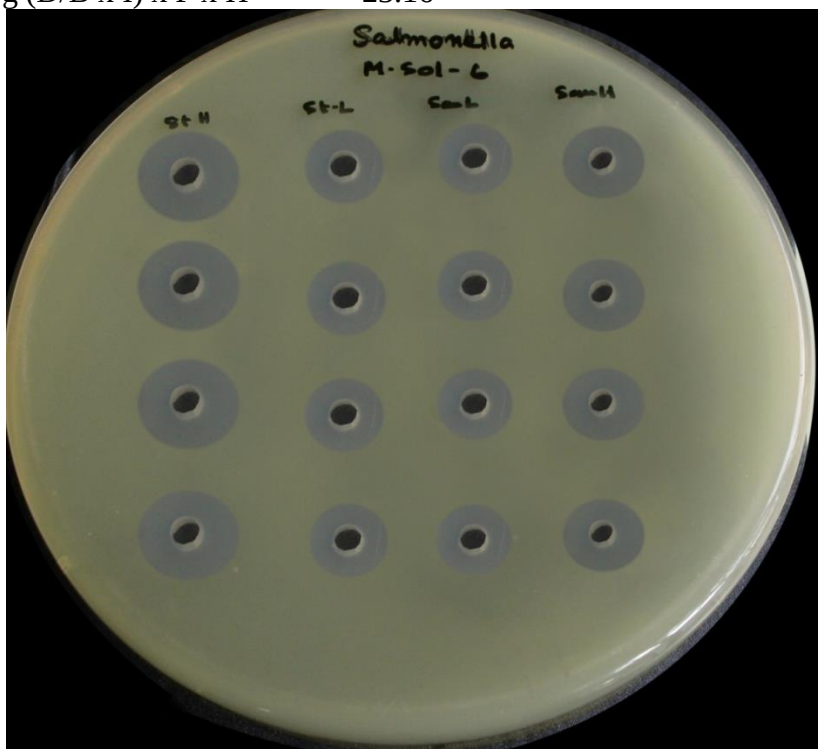


Fig-3.16 Biopotency of Merc-sol-6against *Salmonella typhi*

Table 3.18.Biopotency of Sulpher-6 against S.*typhi*

	Sulfur-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
S. typhi	17.2	17.1	20.1	20.2	19.6	19.5	*22.1*	22.2
	17.3	17.2	20.3	20.1	19.4	19.5	*22*	22.2
	17.3	17.1	20.2	20.3	19.6	19.6	*22.2*	22.1
	17.4	17.5	20.1	20.2	19.5	19.5	22.3	22.2
sum	T2	69.5	T1	80.75	S2	78.1	S1	88.65

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	80.75	69.5	149.8	11.7
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	88.65	78.1	166.75	10.55

D=(T1+ T2) – (S1+ S2)=-16.95
B=(T1+ T2) + (S1+ S2)=22.25
D/B= -0.762
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.5898
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 23.59

Fig-3.17 Biopotency of Sulfur-6against *Salmonella typhi*

Table 3.19 .Biopotency of Merc-sol-6 against *Shigella dysenterae*

	Merc-sol-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
S. typhi	10.5	10.52	15.45	15.9	10.33	10.37	22.33	22.35
	10.45	10.53	15.46	15.48	10.32	10.36	22.34	22.33
	10.49	10.55	15.49	15.47	10.34	10.35	22.35	22.34
	10.51	10.49	15.5	15.66	10.34	10.36	22.36	22.31
sum	T2	42.02	T1	62.205	S2	82.09	S1	95.865

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	62.205	42.02	104.225	20.185
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	95.865	82.09	177.955	13.775

D=(T1+ T2) – (S1+ S2)=-73.77
B=(T1+ T2) + (S1+ S2)=33.96
D/B= -2.17
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.244
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 8.96

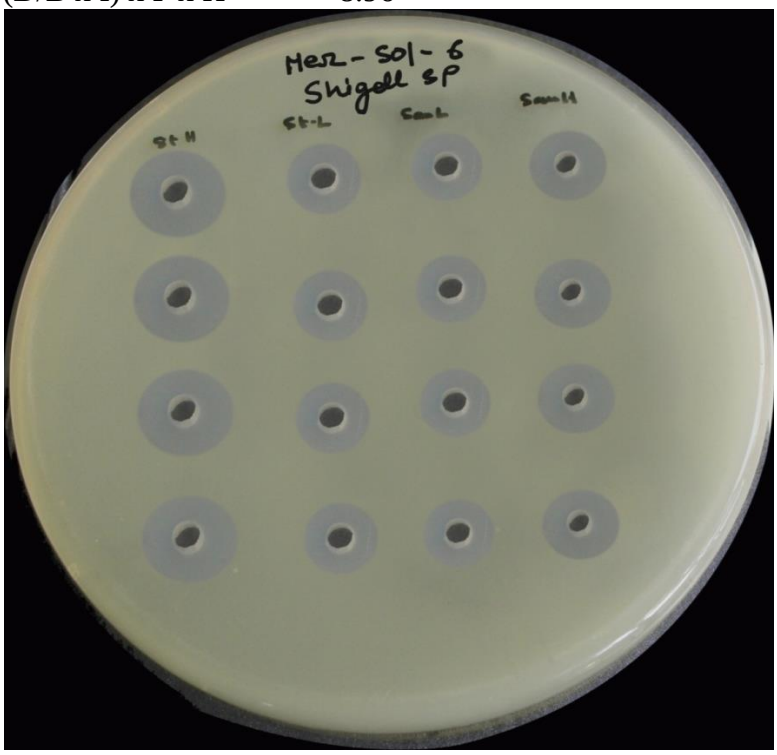


Fig-3.18 Biopotency of Merc-sol-6 against *Shigella dysenteriae*

Table 3.20 .Biopotency of Sulpher-6 against *Shigella dysenterae*

	Sulfur-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
Shigella dysenterae	10.33	10.37	22.33	22.35	10.33	10.37	22.33	22.35
	10.32	10.36	22.34	22.33	10.32	10.36	22.34	22.33
	10.34	10.35	22.35	22.34	10.34	10.35	22.35	22.34
	10.34	10.36	22.36	22.31	10.34	10.36	22.36	22.31
sum	T2	41.385	T1	89.355	S2	82.09	S1	95.865

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	89.355	41.385	130.74	47.95
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	95.865	82.09	177.955	13.775

D=(T1+ T2) – (S1+ S2)=-47.215
B=(T1+ T2) + (S1+ S2)= 61.725
D/B= -0.765
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.588
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 23.52

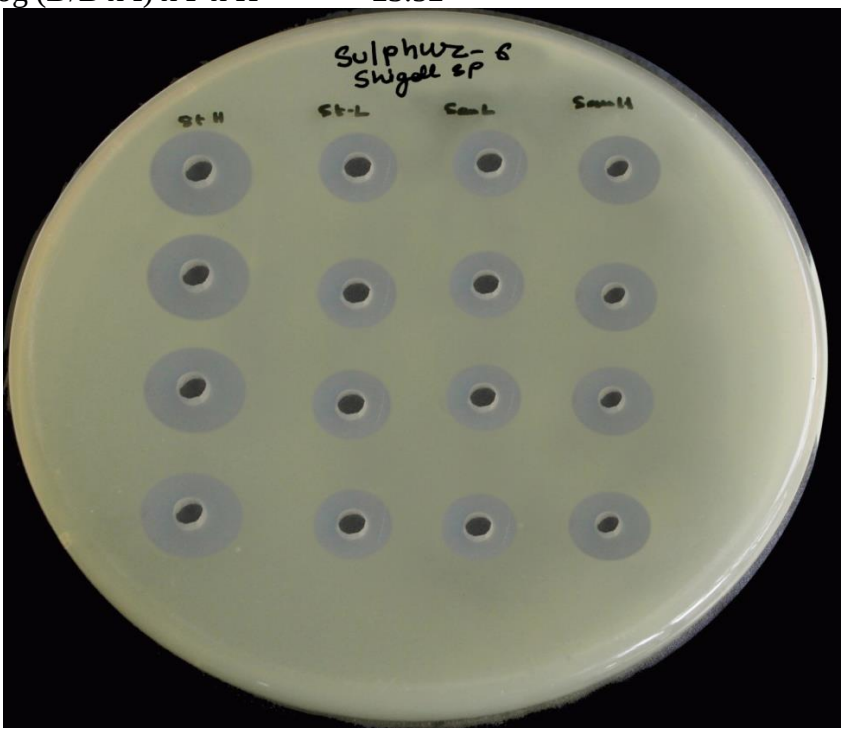


Fig-3.19 Biopotency of Merc-sol-6 against *Shigella dysenteriae*

Table 3.21 .Biopotency of Merc-sol-6 against *Vibrio cholerae*

	Merc-sol-6				Azithromycin			
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
Vibrio cholerae	16.2	16.21	19.46	19.47	20.18	20.17	*24.96*	25.03
	16.3	16.24	19.45	19.45	20.14	20.2	*24.99*	24.97
	16.1	16.33	19.44	19.41	20.15	20.19	*25.01*	25.9
	16.4	16.42	19.42	19.44	20.16	20.18	25.02	25.8
sum	T2	65.1	T1	77.77	S2	80.685	S1	100.84

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	77.77	65.1	142.87	12.67
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	100.84	80.685	181.525	20.155

D=(T1+ T2) – (S1+ S2)=-38.685
B=(T1+ T2) + (S1+ S2)= 32.825
D/B= -1.179
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.446
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 17.84

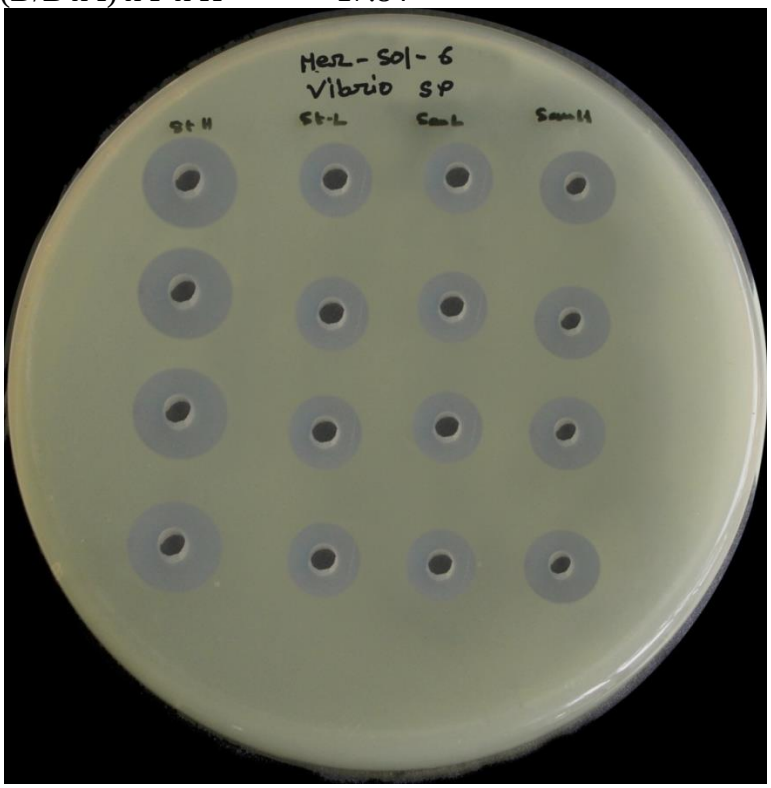


Fig-3.20 Biopotency of Merc-sol-6 against *Vibrio cholerae*

Table 3.22 Biopotency of Sulfur-6 against *Vibrio cholerae*

							ithromycin	
Conc	50µl	50µl	100µl	100µl	50µl	50µl	100µl	100µl
Vibrio cholerae	15.46	15.41	18.09	18.05	20.18	20.17	*24.96*	25.03
	15.43	15.42	18.04	18.04	20.14	20.2	*24.99*	24.97
	15.42	15.44	18.06	18.06	20.15	20.19	*25.01*	25.9
	15.41	15.46	18.02	18.03	20.16	20.18	25.02	25.8
sum	T2	61.725	T1	72.195	S2	80.685	S1	100.84

Used drugs	**T1**	**T2**	**(T1+ T2)**	**(T1- T2)**
Merc-sol-6	72.195	61.725	133.92	10.47
Azithrimycin	**S1**	**S2**	**S1+S2**	**S1-S2**
	100.84	80.685	181.525	20.155

D=(T1+ T2) – (S1+ S2)=-47.605
B=(T1+ T2) + (S1+ S2)= 30.625
D/B= -0.935
I=Log ratio of dilution used=0.301
Antilog (D/B x I)=0.523
F= Factor of dilution used (Highest dilution)= 4
H= Highest dilution used= 10
Potency =Antilog (D/B x I) x F x H = 20.92

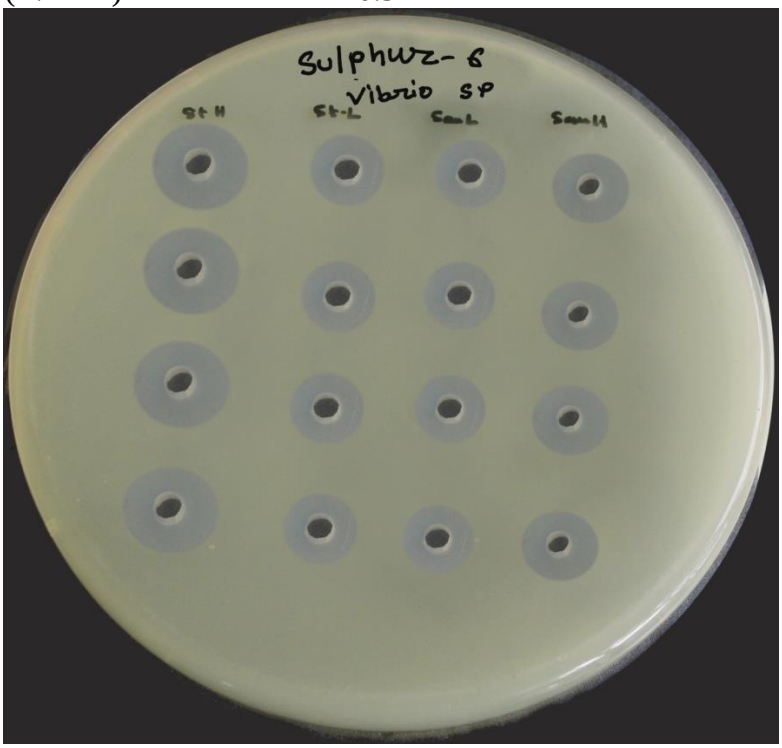
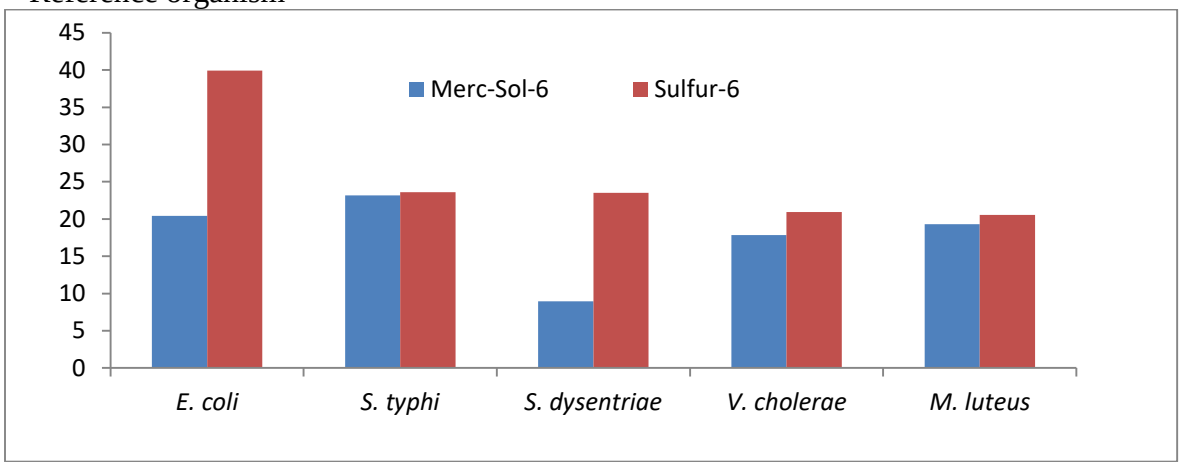


Fig 3.21.Biopotency of Sulfur-6 against *Vibrio cholerae*

3.23 Biopotency of Merc-Sol-6 and Sulfur-6 against enteric pathogens

	Merc-Sol-6	Sulfur-6
E. coli	20.42	39.92
S. typhi	23.16	23.59
S. dysentriae	8.96	23.52
V. cholerae	17.84	20.92
*M. luteus**	19.324	20.56

*Reference organism

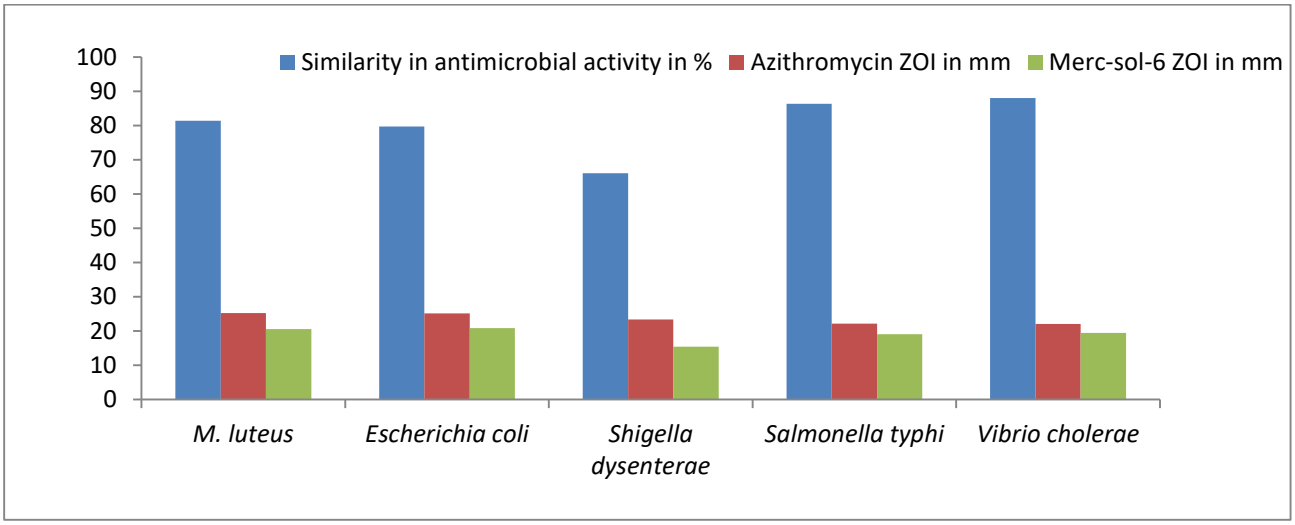


3.5 Relative potency of merc-sol-6 and 7, and sulfur-6 and 7 in comparison to azithromycin

Antibacterial activity of merc-sol-6 and merc-sol-6 and sulfur- 6 and sulfur- 7 were determined comparing to the antibacterial activity of Azithromycin (50 µg/disc). The results are shown in table 12.

Table 3.24a.Relative potencies of merc-sol-6, and sulfur-6 in comparison to azithromycin.

Test organisms	Homoeopathy medicine		Antibiotic	Relative potency of homoeopathy medicines to azithromycin (100%)	
	Merc-sol-6	Sulfur-6	Azithromycin	Merc-sol-6	Sulfur-6
Escherichia coli	20.09±.21	20.13±.3	25.2 ±.1	79.72	79.88
Shigelladysenterae	15.45±.15	22.33±.18	23.38± .12	66.08	95.51
Salmonella typhi	19.13±.3	21.67±.35	22.16±.08	86.33	97.78
Vibrio cholerae	19.46±.2	18.05±.08	22.1±.06	88.05	81.67



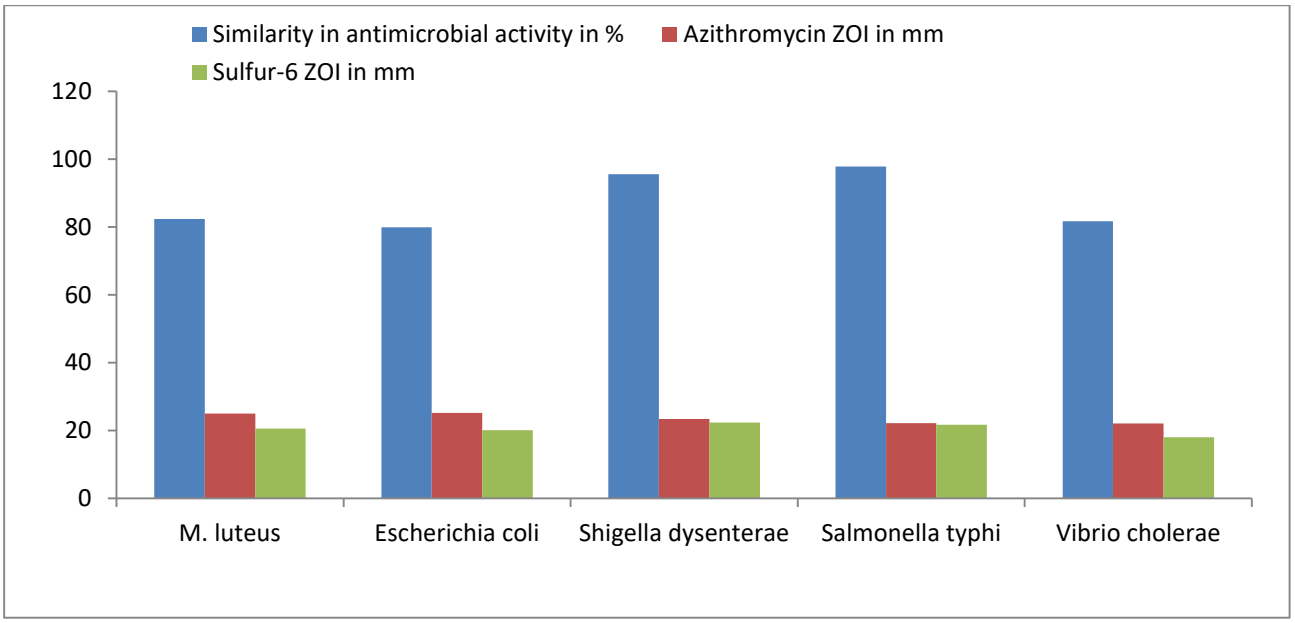


Table 3.24b.Antibacterial Relative Activity of Merc-sol-7 (dynamized from Merc-sol-6) in comparison to merc-sol-6

	Merc sol-6 100ml	Merc sol-7 100ml	Antibacterial Relative Activity Merc sol-7	% of activity decreased
Escherichia coli	20.09±.21	11.04±.01	54.95%	45.05
Shigella spp.	15.45±.15	10.25±.06	66.34%	33.66
Salmonella typhi	19.13±.3	10.13±.3	52.95%	47.05
Vibrio spp.	19.46±.2	10.21±.2	52.47%	47.53
Micrococcus luteus	24.67±.34	10.36±.02	41.99%	58.01

Table 3.24c.Antibacterial Relative Activity of Sulfur-7 (dynamized from Sulfur-6)

	Merc sol-6 100ml	Merc sol-7 100ml	Antibacterial Relative Activity Merc sol-7	% of activity decreased
Escherichia coli	20.13±.3	11.04±.4	54.84%	45.16
Shigella spp.	22.33±.18	10.33±.08	46.26%	53.74
Salmonella typhi	21.67±.35	9.37±.06	43.24%	56.76
Vibrio spp.	18.05±.08	11.05±.08	61.22%	38.78
Micrococcus luteus	24.13±.20	10.13±.03	41.98%	58.02

CHAPTER: 04

DISCUSSION AND CONCLUSION

Since beginning of homoeopathy the high dilution form of the homoeopathy drugs is discussion theme of their activity. Many theories are present to show the activity of homoeopathy drugs. **1.** Vital force theory **2.** Storing energy Dr Benvenistye- Theory of the memory of water Homoeopathy medical stream proves their utility in all type of illness.[99]

The study revealed that out of 5 homoeopathic drugs,2 showed satisfactory antimicrobial activity against enteric pathogens. Five homoeopathy medicines were used such as Mercurius Corrosivus-6 (Merc Cor-6), Mercurius Solubilis-6 (Merc Sol-6), Pyrogenium-6, Hepar Sulfuris calcareum-6 (Hepar Sulp-6), and Sulfur-6.

These all medicines were applied against *Salmonella typhi*, *E. coli*, *Shigella dysentriae, Vibrio cholerae, Bacillus cereus and Micrococcus luteus*. Merc-sol-6 and Sulfur-6 both of the medicines showed zone of inhibition against *Salmonella typhi*. and *E.coli* . Based on sensitivity to all five homoeopathy medicines *Micrococcus luteus* was selected as reference organism for further study.

At the beginning of research work, disk diffusion method was used by using sterile filter paper, and inoculation was done by swabbing using Kirby Bauer method. Primary Antibiogram method exhibited enhanced growth and lower antimicrobial activity. Later agar well method was used for better results, and less contamination and better results were obtained. Finally, disk diffusion method was again used by using blank sterile antibiotic disks . And plates were inoculated by pour plate method to obtained confluent growth and far-clear zone of inhibition.
Five Homoeopathy medicines were used such as Mercurius Corrosivus-6 (Merc Cor-6), Mercurius Solubilis-6 (Merc Sol-6), Pyrogenium-6, Hepar Sulfuris calcareum-6 (Hepar Sulp-6), and Sulfur-6. All these medicines are applying against *Salmonella typhi*, *Escherichia coli*,

Shigella spp., Vibrio spp. and Micrococcus luteus. Merc-sol-6 and Sulfur-6 both of medicines show zone of inhibition against *Salmonella typhi* and *Escherichia coli* .

Merc-sol-6 showed zones of inhibition of 13.1±0.20/50 µl/disc and 20.09±0.21/100 µl/disc; 10.5±0.33/50 µl/disc and 15.45±0.15/100 µl/disc; 15.3±0.18/50 µl/disc and 19.13±0.30/100 µl/disc; 14.33±0.24/50 µl/disc and 19.46±0.20/100 µl/disc against *E. coli O157:H7 ,Shigella dysenterae , Salmonella typhi , Vibrio cholera*. On the other hand sulfur-6 showed zones of inhibition of 12.67±0.31/50 µl/disc and 20.13±0.30/100 µl/disc; 10.33±0.20/50 µl/disc and 22.33±0.18/100 µl/disc, 14.33±0.28/50 µl/disc and 21.67±0.35/100 µl/disc and 9.13±0.20/50 µl/disc and 18.05±0.08/100 µl/disc against *E. coli O157:H7 ,Shigella dysenterae , Salmonella typhi and Vibrio cholera* respectively.

MIC and MBC of the active Homoeopathy drugs were carried out by broth dilution method. MIC and MBC values were found ranged between 64-128 µl, and 128-256µl, respectively. MIC and MBC was done by broth dilution method where most of the organism exhibited their inhibitory activity around 128mg, and bacteriocidal activity at 256mg. Merc-sol-6 displayed a strong antimicrobial activity with MIC and MBC values as low as 64 µl/ ml and 128 µl/ml, respectively against *E. coli O157:H7* and *Salmonella typhi* and 226 µl/ ml and 512 µl/ml, respectively against *Shigella dysenterae* and *Vibrio cholera.* In many similar works, MIC and MBC revealed only inhibitory activity and weak interaction of homoeopathy drugs against pathogens.[100]

Merc-sol - 6 exhibited highest relative activity followed by Sulfur - 6. Antimicrobial activity of Sulfur 6 against *E. coli* was 91.31% , against *Salmonella typhi* was 91.11% , against *M. luteus* was 82.28% compared to the relative antibacterial activity of Azithromycin. Where similarity of of Merc-sol 6 against *E. coli* was 80.33% , against *S. typhi* was 86.52% and against *M. luteus*

was 81.36% compared to the relative antibacterial activity of Azithromycin. Bio-potency of merc-sol-6 and sulfur-6 was determined in comparison to azithromycin using modified method of determining biopotency used by Samsul et. al. [97] . Bio-potency of merc-sol-6 exhibited 19.33%, 20.42%, 8.96%, 23.16%, and, 17.84% against *M. luteus, E. coli O157:H7, Shigella dysenterae, Salmonella typhi,* and *Vibrio cholerae,* respectively . Similarly Biopotency of sulfur-6 revealed 20.56%, 39.92%, 23.52%, 23.59% and 20.88%, potency against *M. luteus, E. coli O157:H7, Shigella dysenterae, Salmonella typhi,* and *Vibrio cholerae* respectively.

Merc-sol-6 and sulfur-6 were dynamized to merc-sol-7 and sulfur-7 and their antibacterial activity was determined as per suggestion given during seminer in Department of Microbiology, University of Dhaka. Merc-sol-7 showed zones of inhibition of 9.40±0.10/50 µl/disc and 11.04±0.01/100 µl/disc; 8.5±0.34/50 µl/disc and 10.25±0.06/100 µl/disc; 8.23±0.12/50 µl/disc and 10.13±0.31/100 µl/disc and 9.34±0.12/50 µl/disc and 10.21±0.20/100 µl/disc respectively against *E. coli O157:H7 ,Shigella dysenterae , Salmonella typhi and Vibrio cholera* . Whereas sulfur-7 showed zones of inhibition of 9.37±0.01/50 µl/disc and 11.04±0.40/100 µl/disc; 8.35±0.02/50 µl/disc and 10.34±0.08/100 µl/disc; 8.33±0.08/50 µl/disc and 9.38±0.06/100 µl/disc and finally 8.23±0.20/50 µl/disc and 11.05±0.08/100 µl/disc against *E. coli O157:H7 ,Shigella dysenterae , Salmonella typhi* and *Vibrio cholera* respectively. These findings showed a little less activity than merc-sol-6 and sulfur-6 reduction in zone of inhibition ranged from 33.66% (*Shigella dysenterae*) to 47.53% (*Vibrio cholera)* in case of merc-sol-6 and 38.78% (*Vibrio cholera*) to 56.76% (*Salmonella typhi*).

In homoeopathy potentization process, probability of finding even a single molecule of original drug is nil (after 24X or 12C but similar studies revaled that it is enough to inhibit growth of

bacteria and this study revealed that homoeopathy drugs are capable of exhibiting bactericidal activity.[100] Mechanism of homoeopathy medicine are belived to be one of this 2 hypothesis,

1. Direct bactericidal action may be attributed to the property of alcohol to dehydrate cells.[100] However, during antibiogram against pathogens, Rectified spirit of same concentration were used as negative control same as the concentration used during manufacture of the medicine, which revealed no antimicrobial activity, where two test drugs showed significant antimicrobial activity in comparison to rectified spirit.

2. A very weak antibiotic activity is probably due to similarity of the medicine of particular potency which resonantly matches with bacteria not in structure but in pathogenic power and hence creating environment surrounding bacteria and hence interfering with nutritional uptake by bacteria consequently causing disturbance in metabolism, protein synthesis, information transfer and eventually causing death of bacteria.[100]

It has also been reported that Homeopathic medicines are used in infectious conditions not in the manner of antibiotics as they don't directly kill the bacteria but rather inhibit the growth of the bacteria while potency of drugs were 6CH, 12CH 30CH, 200CH, 1M, 10M, 50M.[101]However present study revealed bactericidal activity of Homoeopathy medicine against test organisms. Different potency of medicine should be used in future for further study and better understanding for mechanism of homoeopahy medicine.

In study of Amir et. al revealed that its moderate activity against *S. aureus*, *P. aeruginosa* and *E. coil*; less activity was observed against *B. subtilis*, *S. typhi* and ineffective against *S. sonnei* bacterial strain at 200 μg/ml, at higher concentration like 400 μg/ml and 800 μg/ml concentration

against *S. aureus, P. aeruginosa* and *E. coli* and moderate activity was observed against *B. subtilis, S. typhi* and *S. sonnei* bacterial strains.[102]In another study homoeopathic mother tinctures proved to be effective in inhibiting the in vitro growth of three clinically important skin pathogenic bacteria, *Staphylococcus aureus, S. pyogenes, and P. aeruginosa.* [103]

In present study, microorganisms showed zone of inhibition at 50 to 100ml of drug when used against inoculums of bacterial suspension against Merc-sol-6 and Sulfur-6.

Merc-sol-6 exhibited highest biopotency, followed by Sulfur 6. Whereas Heper Sulfur 6 was least biologically active.

Conclusion

Five Homoeopathy medicines were used such as Mercurius Corrosivus-6 (Merc Cor-6), Mercurius Solubilis-6 (Merc Sol-6), Pyrogenium-6, Hepar Sulfuris calcareum-6 (Hepar Sulp-6), and Sulfur-6.

All these medicines were applying against *Salmonella typhi, Escherichia coli* 0157:H7, *Shigella dysenteriae, Vibrio spp. and Micrococcus luteus.* Merc-Sol-6 and Sulfur-6 both of the medicines show zone of inhibition against *Salmonella typhi* and *Escherichia coli* . MIC and MBC of the active Homoeopathy drugs were carried out by broth dilution method . MIC and MBC values were found ranged between 64-128 µl, and 128-256µl, respectively. Merc-sol - 6 exhibited highest biopotency, followed by Sulfur - 6.

Antimicrobial activity of Sulfur 6 against *E. coli* 0157:H7 was 91.31% compared to the relative antibacterial activity of Azithromycin, against *Salmonella typhi* it was 91.11% compared to the relative antibacterial activity of Azithromycin and against *M. luteus* was 82.28% .whereas activity of Merc-Sol 6 against *S. typhi* was 86.52% compared to the relative antibacterial activity of Azithromycin and against *M. luteus* it was 81.36% .

Chapter-5

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Appendix 1:

Trypticase soy agar
15 g tryptone
5 g "soytone" – enzymatic digest of soybean meal
5 g sodium chloride
15 g agar

Trypticase soy broth
17 grams (0.60 oz) of Tryptone
3 grams (0.11 oz) of Soy
5 grams (0.18 oz) of NaCl
2.5 grams (0.088 oz) of dipotassium phosphate (K_2HPO_4)
2.5 grams (0.088 oz) of glucose
1 liter (35 imp fl oz; 34 U.S. fl oz) of distilled water

Müller-Hinton agar
2.0g beef extract
17.5g casein hydrolysate
1.5g starch
17.0g agar
dissolved in 1 liter of distilled water.
pH adjusted to neutral at 25 °C.

Physiological Saline:
9 g of NaCl per liter of Distill water

Phosphate buffer Saline:

Component	Mass	Molarity
Sodium phosphate dibasic heptahydrate (mw: 268 g/mol)	20.209 g	0.0754 M
Sodium phosphate monobasic monohydrate (mw: 138 g/mol)	3.394 g	0.0246 M