Antibacterial Properties of the Leaves and Roots of *Mimosa pudica*, *Imperata cylindrica* and *Eleusine indica* on *E. coli*, *S. aureus* and *B. subtilis*

A research paper presented to:
Prof. Josette T. Biyo

In fulfillment of the requirement in
Science Research II

Submitted by:
Apolonio, Risa Rhose
Cortado, Hanna
Tomas, Hedarlyn

Philippine Science High School
Western Visayas
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APPROVAL SHEET

The research paper entitled Antibacterial Properties of the Leaves and Roots of *Mimosa Pudica, Imperate cylindrica,* and *Eleusine indica* on *E. coli, S. aureus* and *B. subtilis* submitted by Risa Rhose Apolonio, Hanna Cortado and Hedarlyn Tomas in partial fulfillment of the course requirements in Science Research II, has been examined and approved.

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Date

_____
Prof. Josette T. Biyo
Adviser

This research paper submitted in partial fulfillment of the course requirements in Science Research II is accepted and approved.

_____
Date

_____
Prof. Rebecca V. Yandog
PSHSWV Director

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Introduction

A. Background of the Study

The Philippine archipelago is rich in its floral resources. Abundant rainfall and rich in soil contributed much to the development of the floral resources. More than thousands of plant species can be found here in the Philippines. Some have been known to be useful for food, medicine, and sources of other materials like cloth, fuel, and even simple products like paper. Some of them were noted to have certain characteristics that made them into unique from others.

We have plants that yield products that can be used as substitutes for imported ones. *Strophantus letei* has been studied locally and been found to contain strophantin. Very likely, other endemic species also contain such as glucoside. Stryhnine is obtained from Strycnos nux-vomica, which is an introduced specie (Quisumbing, 1978).

Eight hundred fifty species of medicinal plants were described by Quisumbing in 1978, sixty-three of which were recognized in various pharmacopoeias throughout the world.

Even just a tiny-weenie weed could be useful. It maybe the cure for the cancers and AIDS. We may know some plants here in the Philippines are the only species found in the whole world. We may not know that papaya could cure cancer. If we leave all these plants undiscovered and untouched, then how could we develop our floral resources. How could we gain a better understanding of our Natural resources? We need to explore the wonders of our natural world. There is a need for us to discover more than what we could just see with our naked eye. We need to gain more knowledge about their importance and certain properties.

Bacteria are present anywhere. They thrive in places where food is abundant and where surrounding conditions fits their lifestyles. These bacteria may cause disease such as diphtheria, tuberculosis, and others. They could endanger out life. Some bacteria may live in extremely salty environments. Some normally grow in hot, acidic environments. Bacteria may be classified as the gram-negative bacteria that possess a thin cell wall. These may be the nitrogen-fixing aerobic bacteria, enterobacteria, spirochetes, cyanobacteria, ricketsias, chlamdias, and myxobacteria. Gram-positive bacteria possess a thick cell wall of peptidoglycan. All are nonphotosynthetic, and may produce spores. They include the lactic acid bacteria, streptococci, staphylococci, clostridia, and actinomycetes. Some of these bacteria may develop in less than a minute. Other bacteria include *Escherichia coli*, *Clostridium botulinum* and *Staphyloccus aureus*. These facts about bacteria motivated our group to study for the antibacterial properties of the leaves and roots of *Mimosa pudica, Imperata cylindrica,* and *Eleusine indica*. These weeds were observed to have been abundant in our country. Unless we make use of them, they will remain as weeds growing on different areas.

D. Significance of the Study

The Philippine archipelago is rich in natural resources. Her faunal and floral resources flourish because of nutrients and abundant water supply. Weeds rob the plants with the nutrients necessary for their growth. Farmers have problems on how to eliminate these weeds. However, these weeds may be useful informs.

In many Tagalog provinces according to Guerrero (1978), a decoction of fresh roots of *Imperata cylindrica,* or commonly called *kogon is used in dysentery.* He also states that the fruiting spikes are regarded as vulnerary in decoction and as a sedative when taken internally.

Decoction of *Eleusine indica,* an abundant weed in the Philippines, is used as a diuretic and in dysentery. Menaut (1978), says that in Cambodia, the whole plant, particularly the roots, may be used as sudorific, and for fevers and liver complaints.

Guerrero (1978), reported that the root *of Mimosa pudica* is administered as a diuretic, and is used against dysentery and dysmenorrhea. He further reported that the entire plant in decoction is considered as an alterant and antiasthmatic. The juice is used to impregnate cotton wool for dressing in any form of sinus difficulty.

Our country has a high waste management problem. Bacteria find the waste dumping areas in our country suitable for growing. Despite of the beneficial effects brought by the bacteria, we are aware that they may cause diseases in plants, animals, and humans. There is also a need to get rid of these microorganisms.

These reasons gave us the idea to come up with the study of the effects of the leaves and roots of certain weeds.

E. Scope and Limitations

Our study utilized three kinds of weeds. They are the *Mimosa pudica, Imperata cylindrica,* and *Eleusine indica.* We used the leaf and the root extracts of these weeds. The bacteria we used were taken from the PSHS Sci-Res Laboratory. Our experiment was conducted at PSHS Sci-Res Laboratory. Our plant samples were collected at different areas in PSHS-WVC.

F. Definition of Terms

1. **bacteria** - most common name for a vast group of one-celled microscopic organisms that encompasses the smallest, simplest, and perhaps first form of cell life that evolved. They are unicellular and furnish both the raw material and the chemical machinery for their own reproduction.

2. **weed** - any plant growing where it is not desired especially a wild plant growing in ground that is under cultivation.

3. **roots** - the usually underground portion of a plant that serves as support, draws food and water from the surrounding soil, and stores food.

4. **culture** - the cultivation of tissues or microorganisms in prepared media or a product of this.

5. ***Imperata cylindrica*** - an erect grass, 30 to SO cm high, the stems solid, rather slender, the nodes breaded. Leaves flat linear-lanceolate , acuminate erect, 20 to 50 cm long, 5 to 9 mm wide. Panicles exerted, dense, subcylindric, spike-like, white, 10 to 20 cm long, 5 to 15 cm in diameter, silvery-silky. Callus hairs copious, about twice as long as the glumes. Spichelets 3 to 4 mm long.

6. ***Mimosa pudica*** - diffusely spreading herb. Stems branched up to 1m. in length, prickly and with numerous hairs. Leaves very sensitive, both the pinnae and leaflets folding when touched. Leaflets narrowly oblong, 1 to 1.5 cm. long, sessile. Flowers pink and very numerous, long peduncled, solitary or 2 to 3 in. each axil, nearly 1cm. in diameter. Peds are flat, slightly curved, numerous, 1 to 2 cm. long, made up of 3 to 5 one-seeded points.

7. ***Eleusine indica*** - a rather stout, tufted annual, erect, glabrous 10 cm to 1 m high. Leaves 10 to 30 cm long; sometimes involute when dry, 3 to 7 mm wide, distichous

rather flaccid, the sheath flattened, spikes 3 to 6, all in a terminal wheerl, or 1 to 2 leaves down, 2.5 to 10 cm long, 3 to 5 mm thick. Spichelets, numerous, crowded, 3 to 5 flowered, 3 to 4 mm long, first glume 1-nerved small, the second, 3-nerved, the third and succeeding ones, ovate, acute.

8. **leaves** - lateral organ evolved from the stem or axis of a vascular plant. Leaves that have not undergone modification for specialized purposes normally consist of two main parts: a stalk and a blade. The green color of the blades of most foliage leaves is due to the presence of chlorophyll, a substance used by the plant to manufacture carbohydrate sugars from water and carbon dioxide. Leaves are the principal organs of food making in vascular plants.

9. ***Escherichia coli*** - pathogens that inhibits the intestinal tracts of humans and other animals as part of the normal microorganism population. Certain strains of *E. coli* can cause moderate to severe diarrhea.

10. ***Staphylococcus aureus*** - the most pathogenic of the staphylococci that causes boils and skin infections and may infect wounds. Certain strains of *S. aureus* cause a form of food poisoning, and some cause, toxic shock syndrome.

11. ***Bacillus subtilis*** - a member of the genus Bacillus that have centrally located endospores and grow at moderate temperature. It is commonly found in the air and may cause serious endophthalmitis, endocarditis, and even meningitis.

12. **Zones of inhibition** - clear zone or plate where bacteria was eliminated by the antibacterial effect of the crude extracts of *Imperata cylindrica, Mimosa pudica, said Eleusine indica.*

Chapter II

Review of Related Literature

A. Weeds

Any plant can be a weed; it depends entirely on where it grows and how man is involved. Basically, a weed is an unintended plant, growing where we do not want it. A tomato in our flower garden is a weed; a zinnia would be a weed in a tomato field. Most weeds today have a worldwide distribution brought about by man.

The escalating use of herbicides has produced a threat to our environment. Herbicides are potentially more dangerous than the herbs themselves. Many of the herbicides are not biodegradable and are dangerous if consumed. Some plants develop genetic weakness after consistent treatment with chemical herbicides.

B. Common weeds used

Mimosa pudica commonly known as *makahiya* is a diffusely spreading herb. Its stems are sparingly prickly and with numerous bristly hairs. Leaves are very sensitive, both the pinnae and leaflets are folding when touched. Leaflets narrowly oblong, 1 to 1.5 cm. long, sessile. Flowers are pink and very numerous, long peduncled, solitary or 2 to 3 in. each axil, nearly 1 cm. in diameter. Peds are flat, slightly curved, numerous, 1 to 2 cm. long, made up of 3 to 5 one-seeded points. Decoction of entire plant is considered antiasthmatic. Root decoction is given in urinary complaints, so as emetic, and also used in aphrodisiac. In large doses, it could be poisonous. Leaf infusion can be used for dysentery, and kidney trouble (de Padua, Lugod, Pancho, 1987).

Imperata cylindrica is found throughout the Philippines in open slopes. It is an erect grass, 30 to 80 cm. high, the stems solid, lather slender, the nodes breaded. Leaves flat linear-lanceolate, acuminate erect, 20 to 50 cm. long, 5 to 9 mm. wide. Panicles exerted, dense, subcylindric, spike-like, white, 10 to 20 cm. long, 5 to 15 cm. in diameter, silvery-silky. Callus hairs copious, about twice as long as the glumes. Spichelets 3 to 4 mm. long it is commonly called as *kogon*. Decoction of fresh roots is used in dysentery and indigestion. The roots and inflorescence are diuretic, restorative, tonic, astringent, and antifebrile; also used for asthma, jaundice, nausea, dropsy due to weakness, and nosebleed (de Padua, et al., 1981).

Eleusine indica is probably the most common of the weeds in the Philippines. It is a rather stout, tufted annual; erect, glabrous 10 cm. to 1 m. high. Leaves 10 to 30 cm. long; sometimes involute when dry, 3 to 7 mm. wide, distichous rather flaccid, the sheath flattened, spikes 3 to 6, all in a terminal wheerl, or 1 to 2 leaves down, 2.5 to 10 cm. long, 3 to 5 mm. thick. Spichelets, numerous, crowded, 3 to 5 flowered, 3 to 4 mm. long, first glume 1-nerved small, the second, 3-nerved, the thud and succeeding ones, ovate, acute. Decoction of the fresh plant is used as a diuretic and in dysentery (de Padua, Pancho, 1989).

C. Microorganisms

Microbiology is a broad term meaning the study of living organism that are individually too small to be seen with the naked eye. It includes the study of bacteria (bacteriology), viruses (virology), yeasts and molds (mycology), protozoa (protozoology), some algae, and some forms of life that do not fit well into any of this groups. Such forms of life are given the name microorganisms. Sometimes they

are called microbes, or in the vernacular, germs (Volk, 1992).

Microorganisms, with vast majority are involved in cycles of nature in which materials are decomposed so they can be used again by the higher forms of life. Others are used in making antibiotics and most played an essential role in food production.

C.I. Bacteria

Almost 100 years ago, the Danish physician Christian Gram developed the gram staining procedure. Bacteria that absorb and retain crystal violet stain during laboratory staining procedures are referred to as gram-positive, whereas those that do not retain the stain are gram-negative. The cell walls of gram-positive bacteria are very thick and consist primarily of peptidoglycan. The cell wall of a gram-negative bacterial cells consist of two layers, a thin peptidoglycan wall, and a thick outer membrane of lipoprotein and lipopolysaccharide.

The difference in the composition of the cell walls of the gram negative bacteria are of great practical importance. For example, the antibiotic penicillin interferes with the peptidoglycan synthesis, ultimately revolting in a fragile cell wall that cannot effectively protect the cell. Penicillin works most effectively against gram-positive bacteria.

Microbiologists recognize three main groups of eubacteria based on differences in cell walls. These are the wall-less bacteria, the gram-negative bacteria and the gram-positive bacteria (Solomon, Berg, Martin, Villee, 1993).

Bacterial pathogens often have special structures or physiological

characteristics that improve the chances of successful host invasion and infection. Virulence factors are structural or physiological characteristics that help organisms cause infection and disease. These factors include structures such as pili for adhesion to cells and tissues, enzymes that either help in invading host defenses or protect the organism from host defenses, and toxins that can directly cause disease.

Bacteria can enter the body by penetrating the skin or mucous membranes, by sexual transmission, by being ingested with food, by being inhaled in aerosols, or by transmission on a *fomite* (any inanimate object contaminated with an infectious agent). If the bacteria are immediately swept out of the body in urine or feces or by coughing or sneezing, they cannot initiate an infection.

A critical point in the production of the bacterial disease is the organism's adherence or attachment, to a host cell's surface. The occurrence of a certain infections depends in part on the interaction between the host plasma membranes and bacterial adherence factors. Adhesins are proteins or glycoproteins found on attachment pili and capsules. Most adhesins that have been identified permit the pathogen to adhere only to receptors on membranes of certain cells or tissues. For example, an adhesin on attachment pili of certain strains of *E. coli* attaches to receptors in certain host epithelial cells. However, very often the capsules and attachment pili are also antiphagocytic structures. It is difficult for phagocytic cells to engulf bacteria that have capsules or attachment pili, so these structures make excellent virulence factors.

Attachment to a host cell surface is not enough to cause an infection. The microbes must also be able to colonize the cell's surface or to penetrate it. Colonization refers to the growth of microorganisms on epithelial surfaces, such as

skin. For colonization to occur after adherence, the pathogens must survive and reproduce despite host defense mechanism. For example, pathogenic bacteria on the skin surface must withstand environmental conditions and bacteriostatic skin secretions.

Coagulase is bacterial enzyme that accelerates the coagulation or clotting of the blood. *S. aureus* produces coagulase to aid in infection. Conversely, the bacterial enzyme streptokinase dissolves blood clots. Pathogens trapped in blood clots free themselves to spread to other tissues by secreting these virulence factors (Madigal, et. al, 1997).

C.2. *Escherichia coli*

Escherichia coli and other bacteria, the protozoan Giardia, and a certain helminths frequently cause lactose intolerance. *E. coli* has significance far beyond its ability to cause diarrhea. It is an important indicator organism because it is always present in water contaminated with fecal material. *E. coli* is usually more numerous than any other organisms and is easier to isolate. Finding *Escherichia coli* in water means that any pathogens found in feces might also be found present.

Escherichia coli also is an extremely versatile opportunistic pathogen - it can infect any part of the body subject to fecal contamination, including the urinary and reproductive tracts and the abdominal cavity after perforation of the bowel. It is present in many bacteremias, cause septicemias, and can infect the gallbladder, meninges, surgical wounds, skin lessions, and lungs, especially in debilitated and immunodeficient patients (Black, 1993).

E. coli is the causative agent in 80% of Urinary Tract Infections (UTIs), but

other enteric bacteria from feces can also cause such infections.

Water usually is tested for fecal contamination by isolating *E. coli* from a water sample. *E. coli* is also called an indicator organism because, as *E. coli,* is a natural inhabitant of the human digestive tract, its presence in water indicates that the water is contaminated with fecal material (Black, 1996).

Escherichia coli has significance far beyond its ability to cause diarrhea. It is an important indicator organism because it is always present in water contaminated with fecal material. *E. coli* is usually more numerous than other organisms and is easier to isolate. Deadly outbreaks of toxin - producing *E. coli* strain 0157:H7 in 1992 and since have been attributed to undercooked hamburgers served at fast - food restaurants. This strain called *E. coli* 0157:H7, unknown only a decade before, was introduced into ground beef during processing, when feces from slaughtered dairy cows was inadvertently mixed with the meat. Public health investigators discovered that 100,000 pound of the contaminated meat was ground and formed into patties. The ground beef contained viable bacteria that could infect a person and cause illness unless the meat was thoroughly cooked. Compared to *Salmonella* and other food-borne pathogens, which require ingestion of thousands of bacteria to produce symptoms, the presence of just a few *E. coli* 0157:H7 organisms can cause overt disease.

It is now estimated that 20,000 cases *of E. coli* 0157:H7 infection occur every year in the United States, and Canadian health authorities claim that the number may be 10 times that high. The deadly *E. coli* strain has also invaded home kitchens, where improperly cooked ground meats load to sporadic outbreaks by disease.

Measures that once controlled such outbreaks by minimizing contamination and by setting cooking temperatures to ensure that dangerous microbes were killed are no longer adequate, according to Morris Potter of the Center for Disease Control. "Something has changed and these measures are no longer able to destroy [the bacteria. "Until the problem can be identified and corrective measures instituted, eating medium rare ground meat will continue to be risky business (Daniel P. Puzo, Los Angeles Times, March 4, 1993, p. H28).

In light of the recent *E. coli* outbreaks, the Food and Dairy Administration (FDA) recommends that hamburger be cooked until the center reaches 86.1 C (155 F) or until the interioris in no longer pink and the juices run clear. These recommendations are especially important to follow in light of new discoveries on the prevalence of the pathogen. *E. coli* 0157:H7 is more common than *Shigella* (the prototype agent of bacterial dysentery) and frequently lives in the intestinal of healthy catties (McKane and Kandel, 1996).

Escherichia coli is a major cause of diarrhea and dysentery - like syndromes that have inspired such imaginative names as "Montezuma's revenge," "turista," "the trots,' and "Delhi Belly." As members) of the normal intestinal flora, most strains of *E. coli* are a virulant and elicit no disease in the gastrointestinal tract. Some strains, however, are pathogenic and cause gastroenteritis. Although a few strains of *E. coli* (called enteroinvasive *E. coli)* can cause local invasive disease of the intestinal epithelium, most pathogenic strains are noninvasive and produce an enterotoxin while growing in the infected persons's intestine. These strains are called enterotoxigenic *E. coli* (ETEC). The toxin is physically and antigenically related to

cholera toxin and stimulates a similar, although milder, watery diarrhea. Although the two toxins are 80 percent identical, their molecular differences are significant - *E. coli* toxin makes people sick, whereas cholera toxin kills.

Unlike cholera, most persons living in endemic regions are resistant to *E. coli* toxin because they have developed antibodies that neutralize it. Endemic regions are usually characterized by poor sanitation, which encourages microbial multiplication in food and water contaminated with feces. The pathogens do not appear to pose a major health threat when they are imported with a returning vacationer, since the unsanitary environment necessary for perpetuating an epidemic does not come home with the traveler. Other strains of the bacterium, called enteropathogenic *E. coli* , cause infectious diarrhea among infants in the United States.

C.3. Bacillus subtilis

Bacillus subtilis is a gram - positive bacterium. They produce agents that inhibit closely related species, called bacteriocins to distinguish them from the antibiotics, which have a wider spectrum of activity. They produce *subtilisin.*

The gram - positive organism, *B. subtilis,* can also be used as a host. *B. subtilis* is not potentially pathogenic, does not produce endotoxin, and secretes proteins into the medium. Although the technology for cloning in *B. subtilis* is not nearly as well developed as that of a *E. coli,* plasmids and phages suitable for cloning have been developed and transformation is a well developed procedure in *B. subtilis.* Disadvantages in using *B. subtilis* as a cloning host exist, however. Plasmid instability is a real problem, and it is hard to maintain plasmid replication over many culture transfers. Also, foreign DNA is not well maintained in *B. subtilis* cells and so

the cloned DNA is often unexpectedly lost. Adapting a bacterium for use as a host for cloning experiment is not always simple (Madigan et. al., 1997).

C.4. *Staphylococcus aureus*

Everyone has had a pimple at some time; most likely it was caused by the *Staphylococcus aureus*, the most pathogenic of the staphylococci. Staphylococcal skin infections are exceedingly common because the organisms are nearly always present on the skin. Strains of staphylococci colonize the skin and upper respiratory tract of infants within 24 hours of birth. (Half of the adult population and virtually all children are carrier of *S. aureus.)* Infection occurs when these organisms invade the skin through a hair follicle; producing folliculities, also referred to as pimples or postules. An infection at the base of an eyelash is called a sty. A larger, deeper, pus-filled infection is a furuncle, boil, or abscess. An estimated 1.5 million Americans have such infections annually. Further spread of infection particularly on the neck and upper back, creates a massive lesion called carbuncle (Black, 1993).

Black (1994) said *Staphylococcus aureus is* a common human pathogen. It is often responsible for skin abscesses and boils. If it invades the blood, it can travel to other tissues and cause pneumonia, meningitis, and osteomyelitis (infection of the marrow cavity of the bones).

The most common food poisoning is caused by the gram - positive coccus *Staphylococcus aureus.* This organism produces several enterotoxins that are released into the surrounding medium or food; if food containing the toxin is ingested, severe reactions are observes within one to six hours, including nausea with vomiting and diarrhea. Six types of *S. aureus* enterotoxin have been identified. A, B, Cl, C2, D, and E. Enterotoxin A is most frequently associated with outbreaks

of staphylococcal food poisoning. The mechanism of action of enterotoxin A is that of superantigen and involves systemic stimulation of large numbers of T-cells. *S. aureus* enterotoxin A is a small single peptide of 30,000 molecular weight that is encoded by a chromosomal gene. Cloning and sequencing of this gene, the *entA* gene, and of several other *S. aureus* enterotoxin genes, show that this family of toxins is genetically related. Although the *entA* gene is chromosomally located, the B and C type *S. aureus* enterotoxin maybe plasmid or transposon-encoded, or alternatively encoded by a lysogenic bacteriophages.

The kinds of foods most commonly involved in *S. aureus* food poisoning are custard- and cream filled baked goods, poultry, meat and meat products, gravies, egg, and meat salads, puddings, and creamy salad dressings. If such foods are kept refrigerated after preparation, they remain relatively safe, as *S. aureus* is unable to grow at low temperatures. However, foods of this type are often not refrigerated, and are frequently kept in warm kitchens or outdoors at summer picnics. Under these conditions, *S. aureus,* which might have entered the food from a food handler during preparation, grows, and produces enterotoxin. Many of the foods involved in staphylococcal food poisoning are not cooked again before eating, but even if they are, this toxin is relatively beatable and may remain active. Staphylococcal food poisoning can be prevented by careful sanitation method or by storage of the food at low temperatures to prevent bacterial growth, and by the discarding of food stored for any period of hours above 4 degree Celsius (Black, 1996).

D. Tetracyclines

The *tetracyclines* display the broadest spectrum of all antibiotics. they are active against both gram-positive and gram-negative bacteria. Because tetracyclines readily penetrate cell membranes, they are the drug of choice for treating infracellular bacterial infections caused by chlamydias, rickettsias, and *Brucella*. Tetracyclines prevent the binding of transfer RNA to 70 S ribosomes.

Since 80 S ribosomes are not affected, the inhibition is specific for prokaiyotes.

Tetracyclines are acid-stable and are readily absorbed from the gastrointestinal tract, so they are administered orally. Milk products and iron-containing foods reduce antimicrobial effectiveness and should be avoided when tetracyclines are taken. The antimicrobial spectrum of tetracyclines is so broad that their extended use disrupts the normal flora and encourages secondary infections by tetracycline-resistant staphylococci or the yeast *Candida albicans* (McKane and Kandel, 1996).

E. Antibiotic Resistance

Chemotherapeutic effectiveness depends upon the sensitivity of the pathogen to the agent. Some microbes respond predictably to certain drugs, making selection of treatment easy. Other microbes may vary in their responses, and laboratory tests are usually required to ensure that the selected therapy is appropriate. Antibiotic resistance, however, may develop in microbes within the population. In fact, the history of chemotherapy has been closely paralleled by the history of drug

resistance. From the early work of Ehrlich to the recent development of zidovudine, microbes resistant to the agents of their potential destruction have emerged.

Antibiotics do not create resistant cells or cause mutations that produce resistant organisms. They do, however, selectively favor the survival and proliferation of drug-resistant strains, which otherwise are only a small subpopulation within the vast majority of sensitive cells. Antimicrobial resistance is acquired either by mutation in the pathogen's chromosome or by direct transfer of R-factor plasmids from antibiotic-resistant strains to sensitive recipients (McKane and Kandel, 1996).

E.1. Mechanism of Antibiotic Resistance

Some microorganisms are naturally resistant to certain antibiotics because they lack the target that the antibiotic affects or because the drug cannot reach its site of action. Fungi, protozoa, and viruses, for example, contain no peptidoglycan and are naturally resistant to penicillin and other inhibitors of bacterial cell wall synthesis. Sensitive microbes, on the other hand, may became resistant to a drug by gaining the ability to

- Inactive or destroy the antibiotic
- Alter their own membranes so they are no longer permeable to the agent
- Alter the target site so it is no longer affected by the drug, or
- Develop a mechanism to bypass the target metabolic reaction.

Chapter III

Methodology

1. Collection of Specimen

1.1 Collection of Test Organisms

The *Staphylococcus aureus, Escherichia colt* and *Bacillus subtilis* strains were taken from the Philippine Science High School -WVC Science Research Laboratory.

1.2 Collection of Plant Specimens

Mimosa pudica, Imperata cylindrica, and *Eleusine indica* were taken from PSHS- WVC . The specimens were immediately brought to the PSHS-WVC Sci-Res Laboratory. They were washed with running water and were rinsed with distilled water.

2. Sterilization of Equipment

All equipment used was washed thoroughly with soap and water. They were sterilized in the oven at 120 C for one hour.

3. Extraction of Crude Extracts

The method used in the extraction was the method used by Prangga and Teran (1998) with some modifications. Wet weight was determined using the weighing scale. Fifty grams of each species of roots and leaves were soaked separately for one hour in 100 ml of distilled water. The specimens were pounded separately. In order to get rid of the suspended solid particles, the crude extracts were filtered several times. They were placed in labeled containers and stored in the refrigerator.

4. Soaking of the Antibacterial Discs of the Crude Extracts

Eight filter paper discs (Whatman #42) were soaked in 50 ml of the aqueous extracts of each specimen for 10 minutes.

5. **Inoculation of Streak Plates**

The method specified in the manual of BBL Products and Laboratory Procedures (1998) in streaking of culture plates was followed. Mueller Hinton Agar (BBL) was used.

First, the entire length of the wire was flamed in incandescence by holding it in 80 degree angle in the outer core of the flame of an alcohol burner. It was performed while quickly moving the wire loop holder down through the flame so that the portion of the holder just above the wire will be flamed slightly. The wire was allowed to cool for a few seconds prior to its contact with the test material.

Next, the cotton plug was removed from the test tube containing the specimen. Then, a part was selected using a purulent appearing material. A thin loop-full of the specimen was removed avoiding contact of the wire with potentially contaminated surface. The cotton plug on the test tube was replaced and placed aside.

The loop was flamed by positioning the end of the wire containing the loop in the cool (blue) core of an alcohol burner for the first several seconds for preheating to avoid spattering of the infectious material prior to complete sterilization in the hotter (yellow) portion of the flame. The wire was allowed to cool.

The plate was rotated by a quarter turn and was streaked again, overlapping the originally streaked area. The loop was flamed and allowed to cool again.

The plate was rotated and the remaining area was streaked again. The plate was returned to its cover on the work surface. The loop was sterilized again. The plate, agar side up was incubated for 24 hours at 37 C.

6. Antibacterial Testing

6.1 Inoculum Preparation

In inoculum preparation, the Bauer Kirby method was revised using the method of Barry (1970).

A visibly turbid suspension was prepared by picking six isolated colonies of the same morphological type from the bacterial culture plates and placing these colonies in five ml BBL Nutrient Broth infusion -The solution was heated at 37 C.

The nutrient and bacteria were mixed. The mixture was compared to the McFarland barium sulfate turbidity standard. It was prepared by adding .5 ml of .048 M Bad [1.175% (wt/vol) $BaCl \cdot 2H_20$] to 99.5 ml of .36 N H_2SO_4, (1% vol/vol).

The nutrient broth and the bacteria were added to Mueller Hinton agar. Four ml of this solution was added to the base layer of Mueller Hinton agar in each petri dish. Three plates were inoculated with each of the test organisms.

6.2. Application of Discs

Within three min. after pouring the agar to the Mueller Hinton Agar, the discs were applied using forceps. Eight discs were applied to each plate. The discs' contents were:

Disc 1: *Mimosa pudica* roots

Disc 2: *Mimosa pudica leaves*

Disc 3: *Imperata cylmdrica* roots

Disc 4: *Imperata cylindrica* leaves

Disc 5: *Eleusine indica* roots

Disc 6: *Eleusine indica* leaves

Disc 7: Positive-control (Tetracycline)

Disc 8: Negative-Control (distilled water)

Within 15 minutes after the discs were applied, the plates were inverted and were incubated at 37 C for 24 hours. The diameter of the clear zones was measured. The measurement was rounded off to the nearest mm using the ruler.

7. Statistical Testing

One-way analysis of variance was used in order to determine if the zones of inhibition were significantly different from each other.

Chapter IV

Results and Discussions

The crude extraction of the roots and leaves of *Mimosa pudica, Imperata cylindrica* and *Eleusine indica* was done at the Philippine Science High School-Western Visayas Campus Science Research Laboratory.

The antibacterial effects of the crude extracts of the plant parts mentioned above were tested on *E. coli,* a common Gram negative bacterium, *S. aureus,* a Gram positive and *B. subtilis,* a Gram positive bacterium also.

Results showed that all plant extracts possessed antibacterial properties on *E. coli.* All discs possessed positive results on *E. coli.* The plant extracts has non-uniform effectiveness as shown by the statistical analysis.

Mimosa pudica leaf extracts has the highest mean of zone of inhibition of 10 mm. Closely following are *Imperata cylindrica* root extracts. *I cylindrica* leaf extracts and *Eleusine indica* leaf extracts which have the means of 9.6 mm, 9 mm, and 7 mm, respectively. The positive control, tetracycline showed a mean of zone of inhibition which is 14 mm.

Mimosa pudica root extracts proved to be least effective among the plant extracts. It has a mean of 6 mm.

The pimple-causing bacteria, *Staphylococcus aureus* inhibited most effectively by the positive control, followed by the root extracts of *Mimosa pudica.* The root extracts of *Mimosa pudica* has a mean of zone of inhibition of 8.33 mm.

The rest of the plant extracts have a common mean of prone of inhibition which is 6 mm.

Imperata cylindrica root extracts has the highest mean of zone of inhibition on *B. subtilis* among the plant extracts. It showed 8 mm of inhibition. Both *I. cylindrica* leaves and *E. indica* leaves have the mean of zone of inhibition of 7 mm. Following are *M. pudica* leaf extracts and *M. pudica* root extracts with means of 6.66 and 6.33 mm, respectively.

Eleusine indica root extract- showed the lowest mean of zone of inhibition which is 6 mm. The positive control tetracycline has the mean of 31.33 mm and negative control has 0 mm.

Imperata cylindrica, Eleusine indica and *Mimosa pudica* were noted for their potential in herbal medicine. They are used as simple remedies for dysentery, kidney complications and as astringents.

Results of the testing confirmed that all crude extracts of plant specimens possessed antibacterial properties on *E. coli, S. aureus,* and *B. subtilis*. The study confirmed the studies done by de Padua, et. al. (1981) that plant specimens used possessed medicinal properties.

Final Testing Results Raw Data

Table 1

Diameter zones of inhibition (mm) in *E. coli* under the different treatments. Values are means of three determinants.

Treatments	Diameter zone of inhibition (mm)
	X ± s.d.
Disc 1	6 ± 0
Disc 2	10 ± .8165
Disc 3	9.67 ± 1.6997
Disc 4	9 ± 2.4495
Disc 5	6.67 ± .9428
Disc 6	7 ± .8165
Disc 7	14 ± .8165
Disc 8	0 ± 0

Table 2

Diameter zones of inhibition (mm) in *S. aureus* under the different treatments. Values are means of 3 determinants.

Treatments	Diameter zone of inhibition (mm)
	X ± s.d.
Disc 1	8.33 ± .4714
Disc 2	6 ± 0
Disc 3	6 ± 0
Disc 4	6 ± 0
Disc 5	6 ± 0
Disc 6	6 ± 0
Disc 7	29 ± 3.6818
Disc 8	0 ± 0

Table 3

Diameter zones of inhibition (mm) in *B. subtilis* under the different treatments. Values are means of 3 determinants.

Treatments	Diameter zone of inhibition (mm)
	X ± s.d.
Disc 1	6.33 ± .4714
Disc 2	6.67 ± .9428
Disc 3	8 ± 1.6330
Disc 4	7 ± .8165
Disc 5	6 ± 0
Disc 6	7 ± 1.4142
Disc 7	31.33 ± 2.4944
Disc 8	0 ± 0

Chapter V

Summary of Significant Findings

1. All crude extracts were able to inhibit the growth of test organisms *Escherichia coli, Staphyloccocus aureus,* and *Bacillus subtilis.* Diameter zones of inhibition was used to determine the antibacterial properties of the extracts.

2. Distilled water that was the negative control showed no zone of inhibition on the three test organisms.

3. One-Way analysis of Variance ($\pm$ 0.05) for all equal sample sizes proved that in every test organisms, zones of inhibition formed by treatments varied significantly ($f < 0.05$).

4. In *E. coli,* mean diameter zones of inhibition of all plant extracts varied from each other significantly. Efficacy of treatments as antibacterial agents could therefore be summarized as follows:

Tetracycline > *Mimosa puaica leaves* > *Imperata cylindnca roots* > *Imperata cylindrica leaves* > *Eleusine indica leaves* > *Eleusine indica roots* > *Mimosa pudica roots* distilled water.

5. In *S. aureus,* mean diameter of zones of inhibition of all plant extracts varied significantly from each other. Efficacy of treatments as antibacterial agents could therefore be summarized as follows:

Tetracycline > *Mimosa pudica roots* {*Mimosa pudica leaves, Imperata cylindrica roots, Imperata cylindrica leaves, Eleusine indica roots* and *Eleusine indica leaves*} > distilled water.

6. In *B. subtilis*, mean diameter of zones of inhibition of all plant extracts did not vary significantly from each other. Efficacy of treatments as antibacterial agents could therefore be summarized as follows:

Tetracycline > *I. cylindrica roots* > *E. indica leaves* *I. cylindrica leaves* > *M. pudica leaves* > *M. pudica roots* > *E. indica roots* > distilled water

Conclusion

All plant extracts possessed antibacterial properties on *E. coli*. Among the root extracts, *Imperata cylindrica* has the highest mean of zone of inhibition. *Mimosa pudica* root extracts has the lowest mean. The leaf extract with the highest mean of zone of inhibition is *Mimosa pudica*. *E. indica* leaf extracts has the lowest.

Data showed that all plant extracts possessed antibacterial properties on *S. aureus*. *M. pudica* root extracts has the highest mean of zone of inhibition. The rest of the root and leaf extracts have the same means.

Imperata cylindrica root extracts has the highest mean of zone of inhibition. *E. indica* root extracts has the lowest. Among the leaf extracts, both *I cylindrica* and *E. indica* leaf extracts have the highest mean. The leaf extract with the lowest mean is that of *M. pudica*.

Leaf extract of *M. pudica* is more effective than its root extracts in inhibiting the test organisms.

Imperata cylindrica root extracts inhibited greater zones of bacteria than *I. cylindrica* leaf extracts.

Leaf extracts of *E. indica* showed greater antibacterial property on the test organisms compared to *E. indica*.

Recommendation

The use of other parts of the tested weeds is highly recommended. Among the parts that must be tested are the flowers, buds, seeds, and stems.

The researchers also recommend the testing of the plant specimen on other gram-negative and gram-positive bacteria. Testing of other species of plant is also advised.

The use of *Mimosa pudica* roots as treatment for *S. aureus* infection, *M. pudica* leaves to inhibit. *E. coli* and *Imperata cylindrica* roots for *B. subtilis* infection.

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Appendix A

Results of Three Replicates at Different Treatment

Diameter zone of inhibition (mm) on *E. coli* under different treatments. Values are of individual replicate.

Treatment	Diameter zone of inhibition (mm)		
	Replicate 1	Replicate 2	Replicate 3
Disc 1	6	6	6
2	11	10	9
3	12	9	8
4	12	6	9
5	8	6	6
6	7	6	8
7	15	14	13
8	6	6	6

Results of Three Replicates At Different Treatments

Diameter zones of inhibition (mm) on *S. aureus* under the different treatments. Values are of individual replicate.

Treatment	Diameter zone of inhibition (mm)		
	Replicate 1	Replicate 2	Replicate 3
Disc 1	9	8	8
Disc 2	6	6	6
Disc 3	6	6	6
Disc 4	6	6	6
Disc 5	6	6	6
Disc 6	6	6	6
Disc 7	34	25	30
Disc 8	0	0	0

Results of Three Replicates At Different Treatments

Diameter zones of inhibition (mm) on *B. subtilis* under the different treatments. Values are of individual replicate.

Treatment	Diameter zone of inhibition (mm)		
	Replicate 1	Replicate 2	Replicate 3
Disc 1	6	6	7
Disc 2	6	8	6
Disc 3	10	6	8
Disc 4	7	6	8
Disc 5	6	6	6
Disc 6	6	6	9
Disc 7	33	28	34
Disc 8	0	0	0

Appendix B

Statistical Testing

Statistical Testing of the Results in *E. coli*

Replicate	*M. pudica* roots	*M. pudica* leaves	*I. cylindrica* roots	*I. cylindrica* leaves	*E. indica* roots	*E. indica* leaves
1	6	11	12	12	8	7
2	6	10	9	6	6	6
3	6	9	8	9	6	8
Σx	18	30	29	27	20	21
	= 145					
Σx^2	108	302	289	261	136	149
	= 1245					
$(\Sigma x)^2 / N$	108	300	280.33	243	133.33	147
	= 1211.667					

Total df = 17

Group df = 5

Error df = 12

Total sum of squares = 76.94444445

Group sum of squares = 43.61111145

Error sum of squares = 33.3333

Source of variation	SS	df	MS
Total	76.94444445	17	
Group	43.61111145	5	8.72222
Error	33.333333	12	2.777775

F computed	3.14
F tabular	3.11
Reject Ho	

Statistical Testing of the Results in *S. aureus*

Replicate	*M. pudica* roots	*M. pudica* leaves	*I. cylindrica* roots	*I. cylindrica* leaves	*E. indica* roots	*E. indica* leaves
1	9	6	6	6	6	6
2	8	6	6	6	6	6
3	8	6	6	6	6	6

Σx	25	18	18	18	18	18
	= 115					
Σx^2	209	108	108	108	108	108
	= 749					
$(\Sigma x)^2$ /N	208.33	108	108	108	108	108
	= 748.33					

Total df = 17

Group df = 5

Error df = 12

Total sum of squares = 14.277778

Group sum of squares = 13.61111108

Error sum of squares = 0.66667

Source of variation	SS	df	MS
Total	14.277778	17	
Group	13.61111108	5	2.72
Error	0.66667	12	0.055

F computed = 49.45

F tabular = 3.11

Reject Ho

Statistical Testing of the Results in *B subtilis*

Replicate	*M. pudica* roots	*M. pudica* leaves	*I. cylindrica* roots	*I. cylindrica* leaves	*E. indica* roots	*E. indica* leaves
1	6	6	10	7	6	6
2	6	8	6	6	6	6
3	7	6	8	8	6	9
Σx	19	20	24	21	18	21
	= 123					
Σx^2	121	136	200	149	108	153
	= 867					
$(\Sigma x)^2$	120.33	133.33	192	147	108	147
	= 840.5					

Total df = 17

Group df = 5

Error df = 12

Total sum of squares = 26.5

Group sum of squares = 7.16667

Error sum of squares = 19.3333

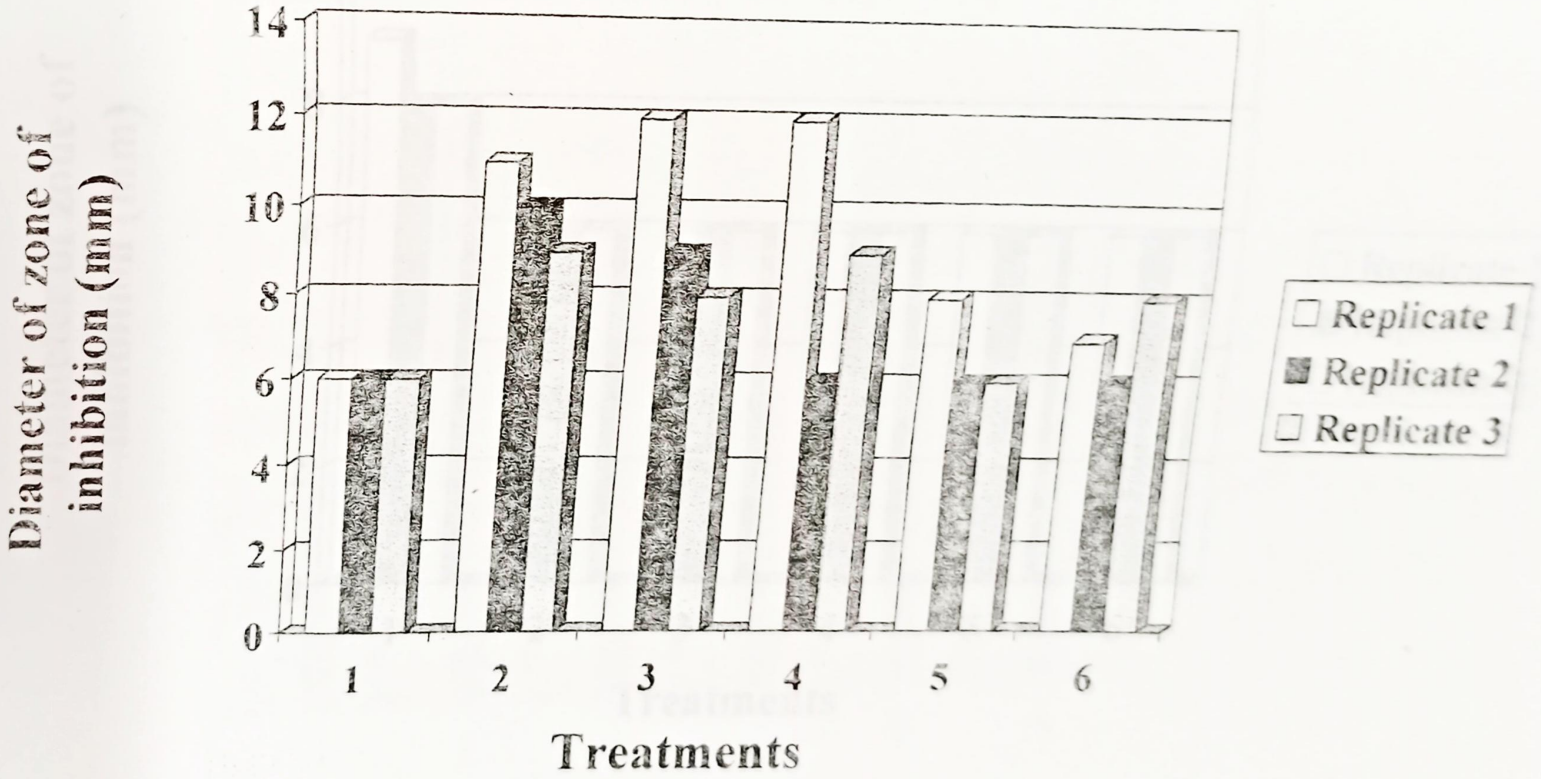
Source of variation	SS	DF	MS
Total	26.5	17	
Group	7.16667	5	1.43333
Error	19.3333	12	1.61111

F computed = 0.889655

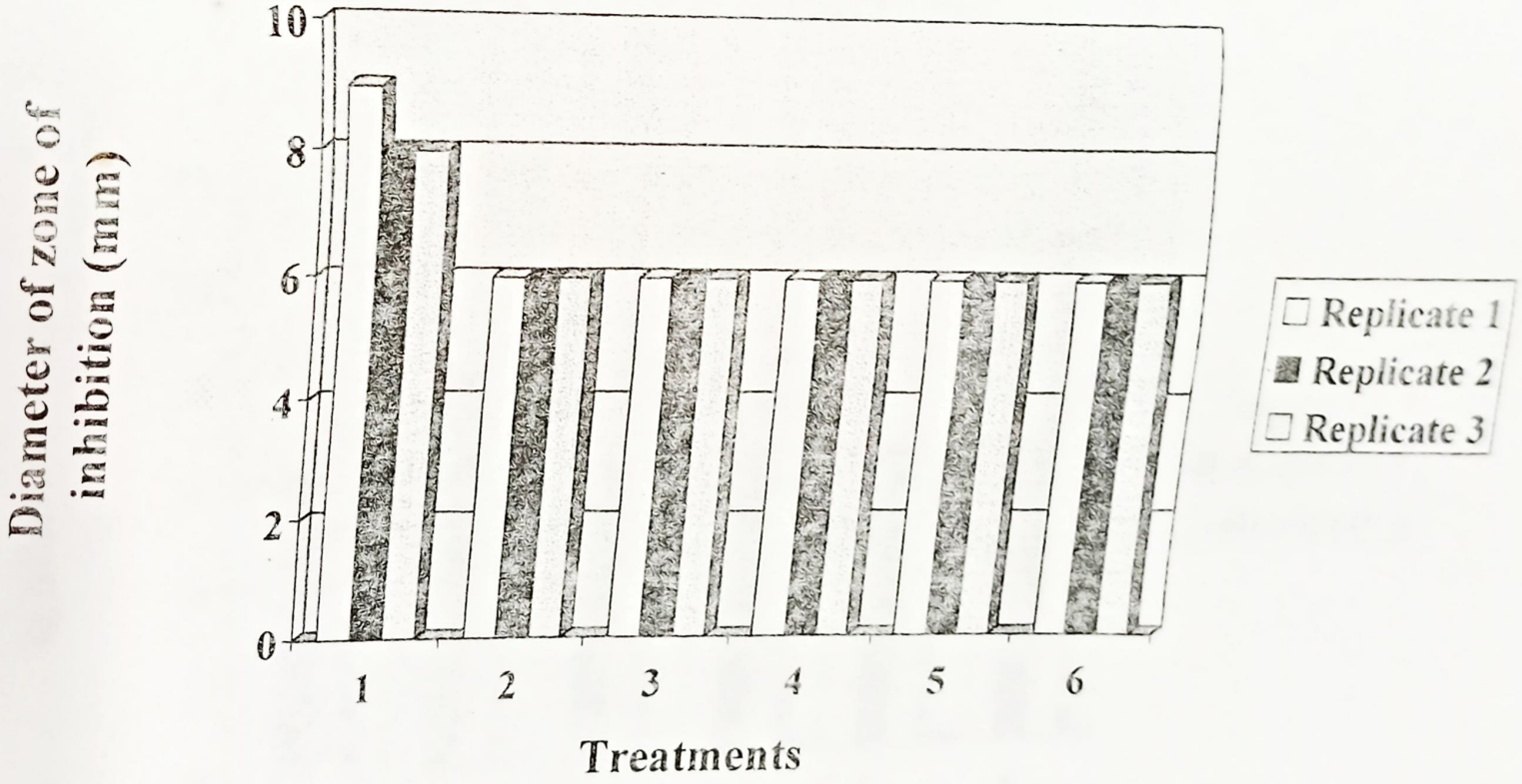
F tabular = 4.68

Accept Ho

The graph of zones of inhibition on *Escherichia coli*

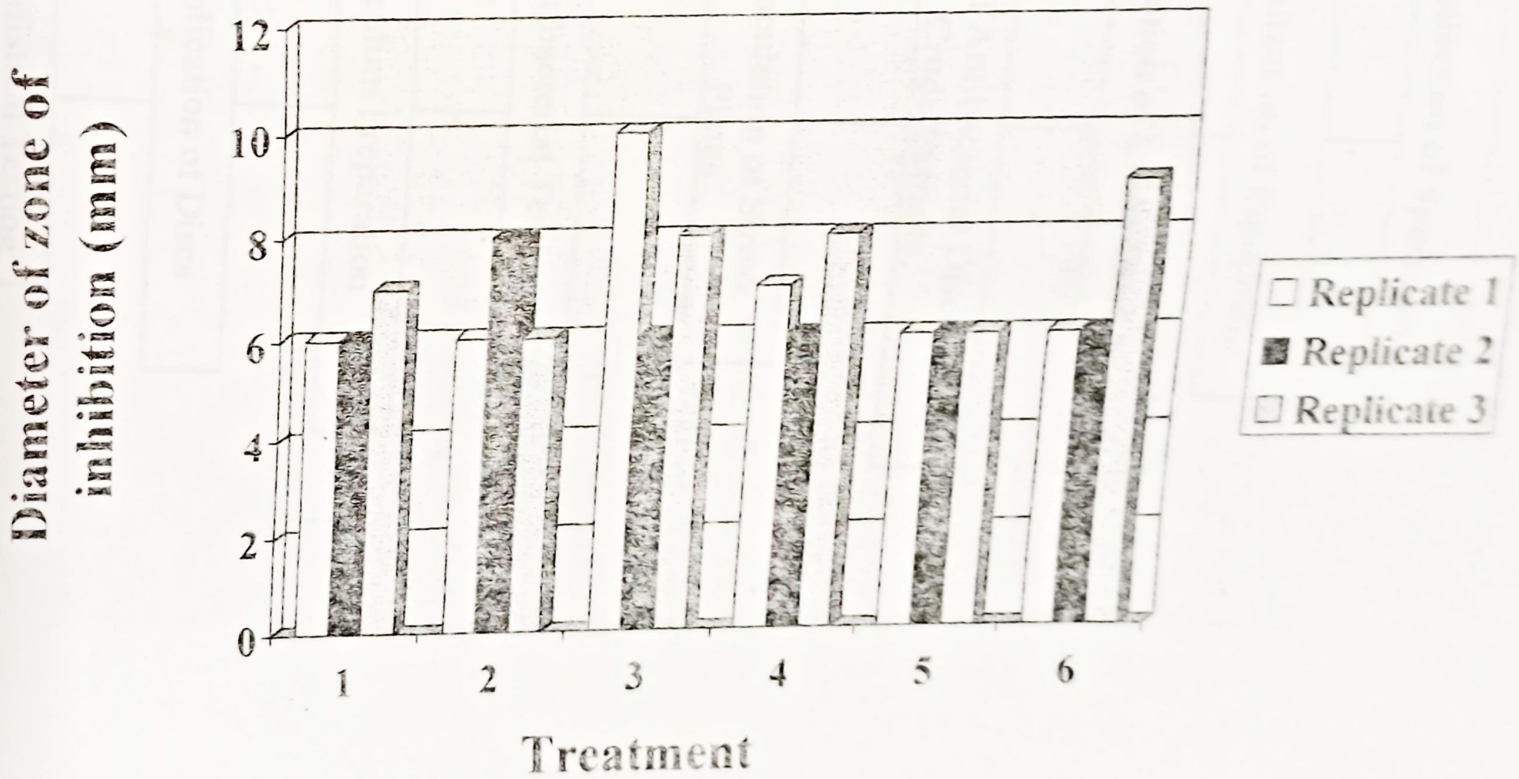


The graph of zones of inhibition on *Staphylococcus aureus*

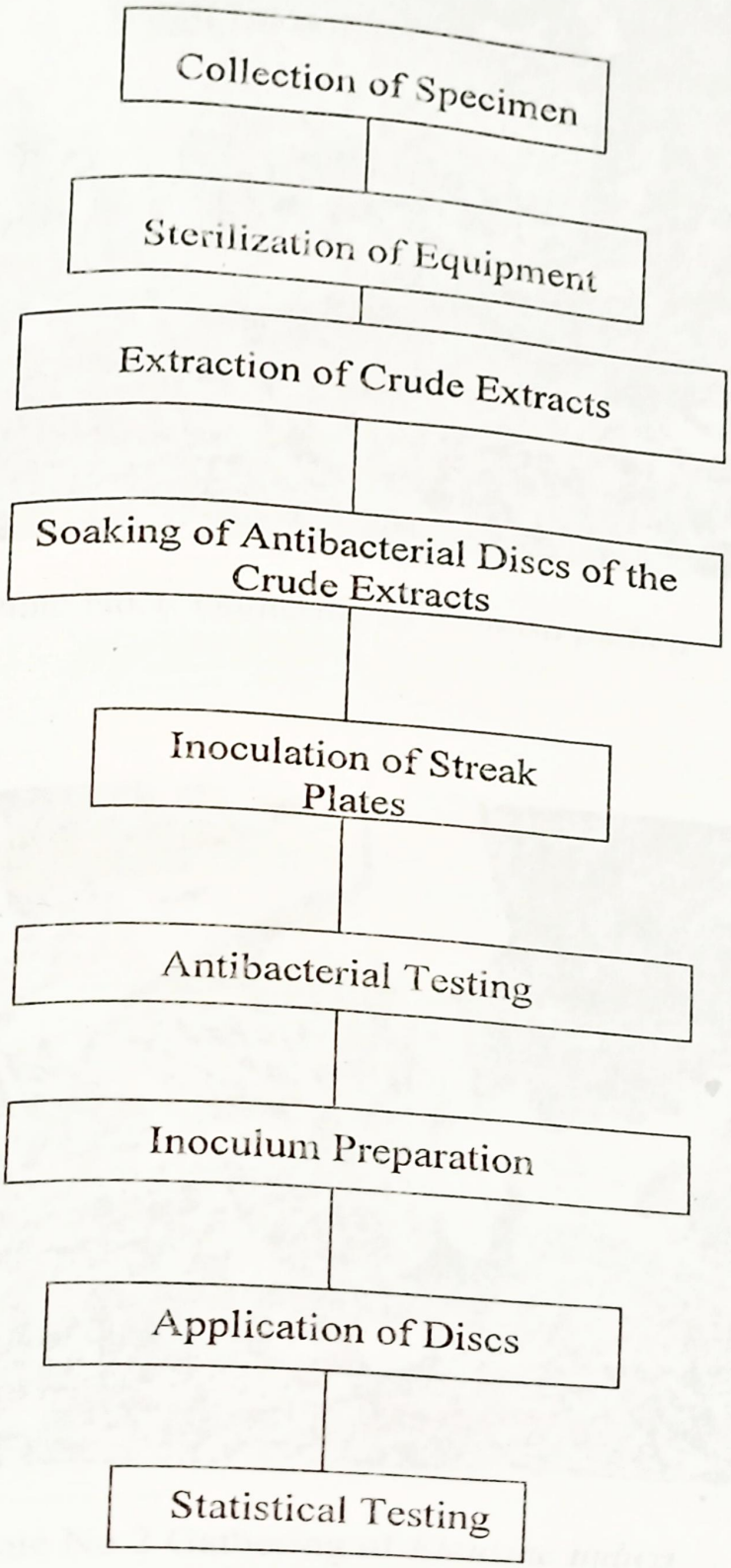


45

The graph of zone of inhibition on *Bacillus subtilis*



Flow Chart


Collection of Specimen
Sterilization of Equipment
Extraction of Crude Extracts
Soaking of Antibacterial Discs of the Crude Extracts
Inoculation of Streak Plates
Antibacterial Testing
Inoculum Preparation
Application of Discs
Statistical Testing

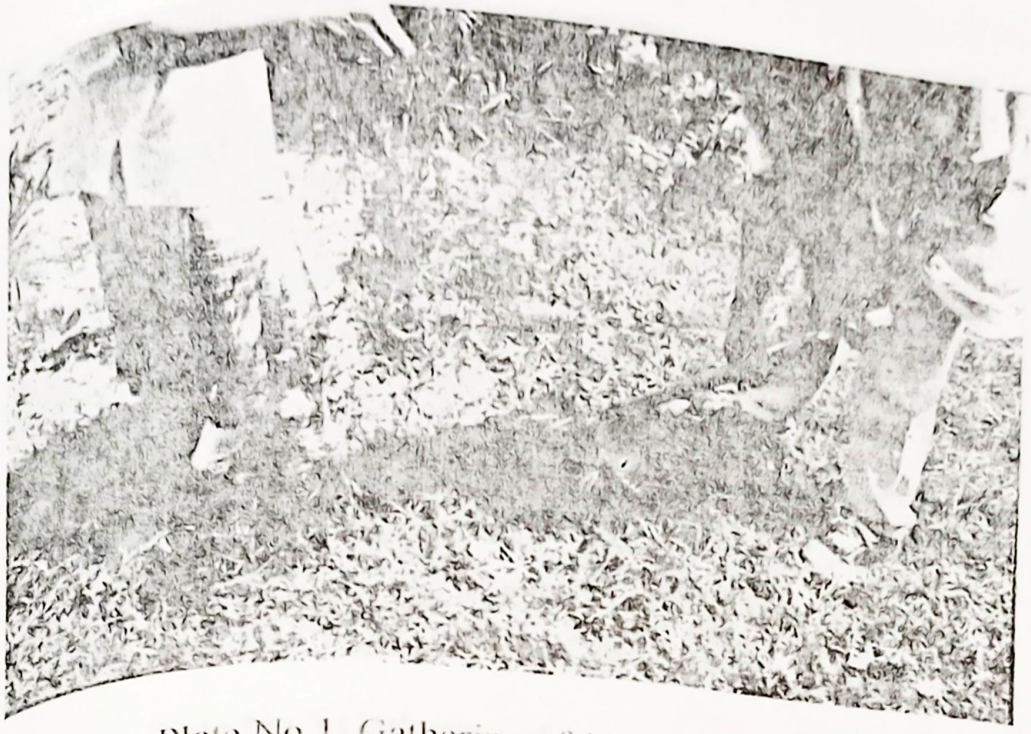


Plate No. 1. Gathering of *Mimosa pudica.*

Plate No.2 Gathering of *Eleusine indica*

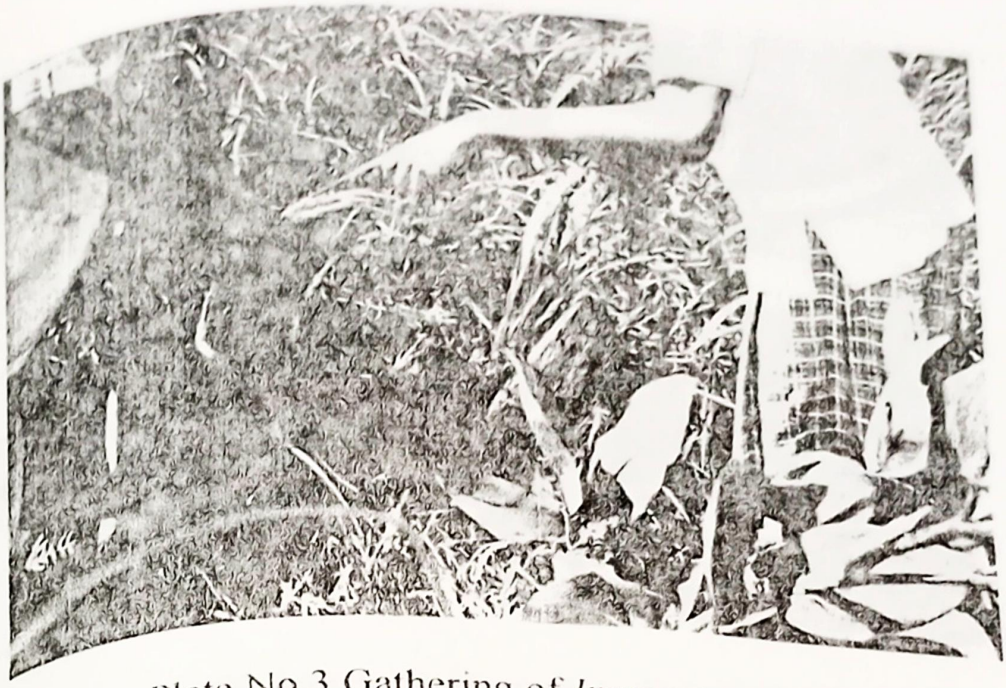


Plate No.3 Gathering of *Imperata cylindrica*

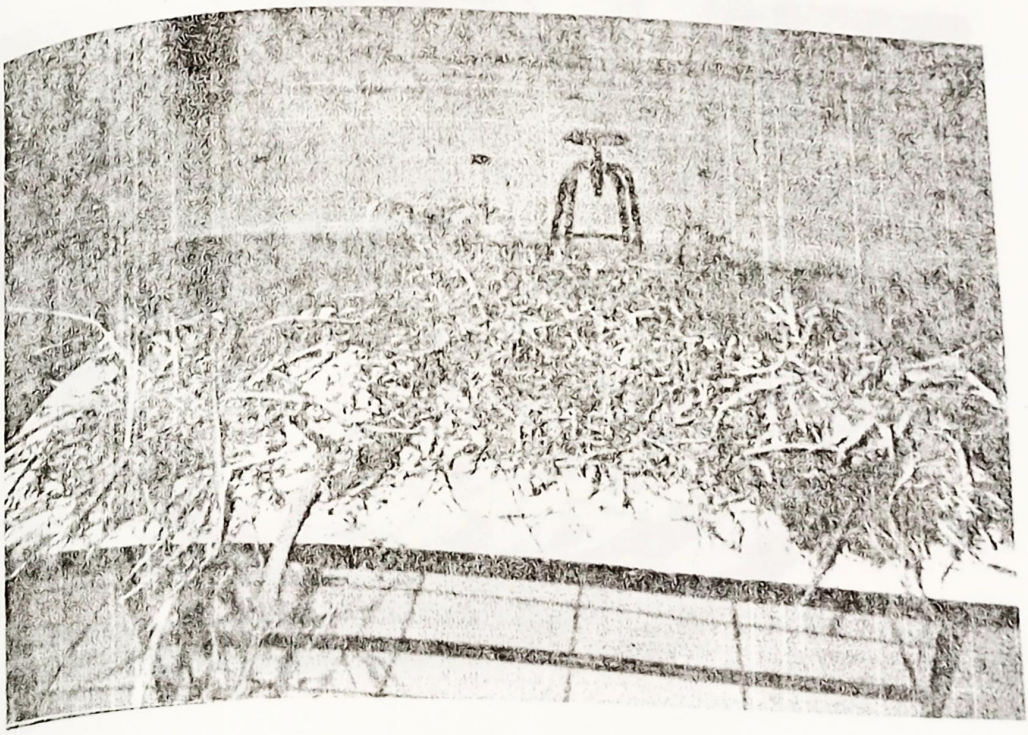


Plate No.4 All weeds needed were brought to the Science Research Laboratory

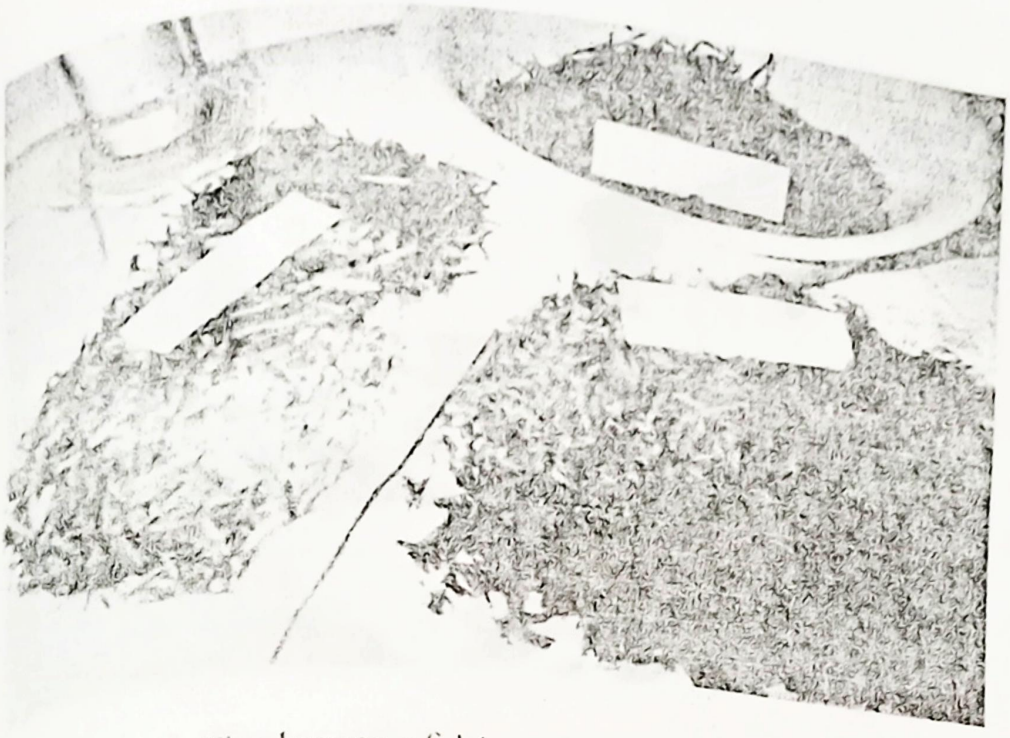


Plate No.5. The leaves of *M. pudica*, *E. indica*, and *I. cylindrica* ready for extraction

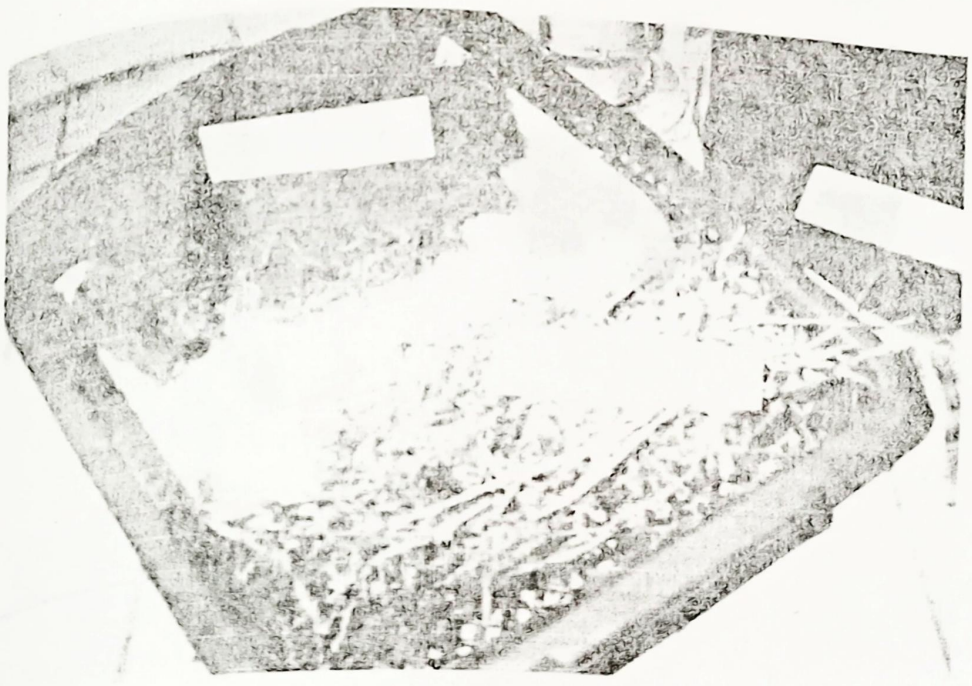


Plate No. 6. The roots of *M. pudica*, *E. indica*, and *I. cylindrica* prepared for extraction

Plate no.7 The extraction of the roots and leaves of chosen species of weeds

Plate No.8. The leaf and root extracts of *M. pudica, I. cylindrica and E. indica.*

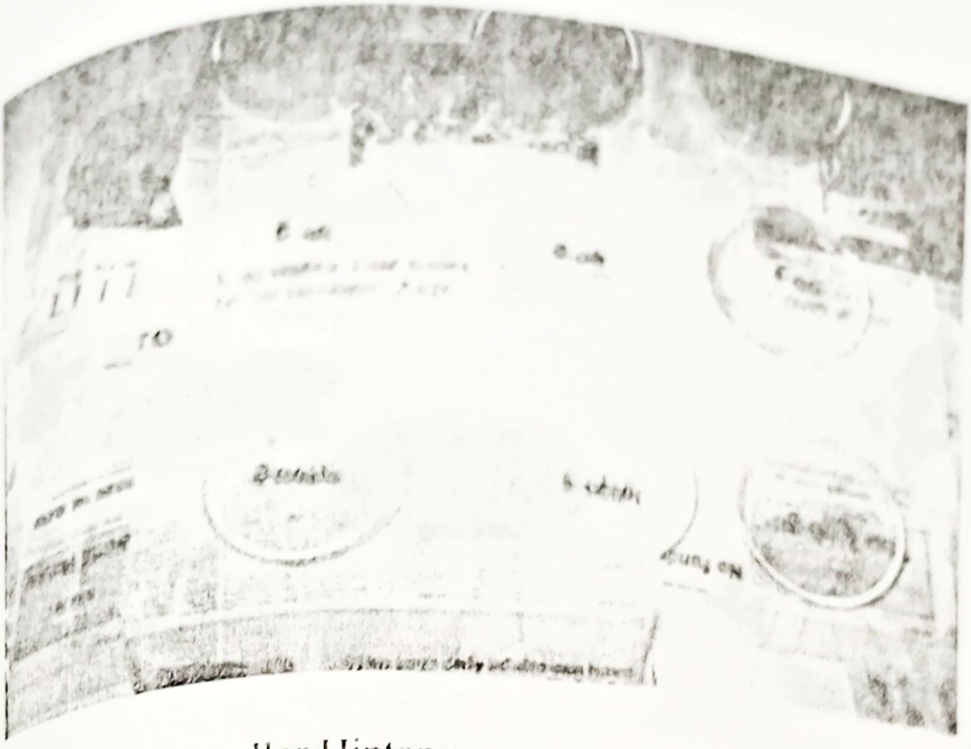


Plate No. 9. Mueller Hinton agar culture media with streaks of *E. coli, S. aureus, and B. subtilis.*

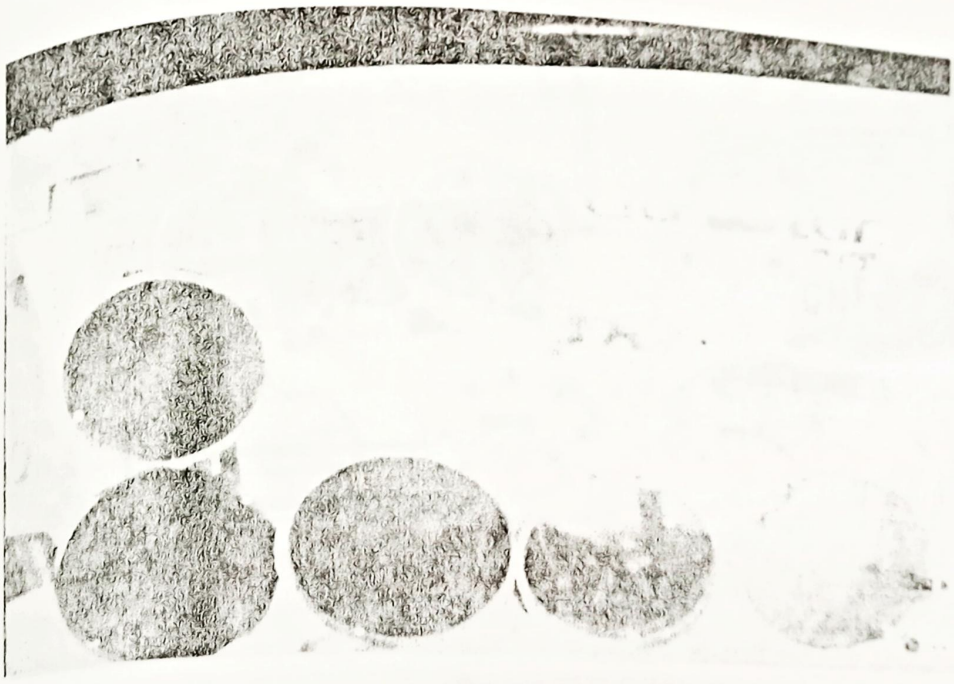


Plate No. 10. Soaking of antibacterial discs on extracts including + and - controls.

Plate No. 11. Antibacterial testing of the crude extracts on *E. coli*, *sincerely. aureus*, *and B. subtilis*.

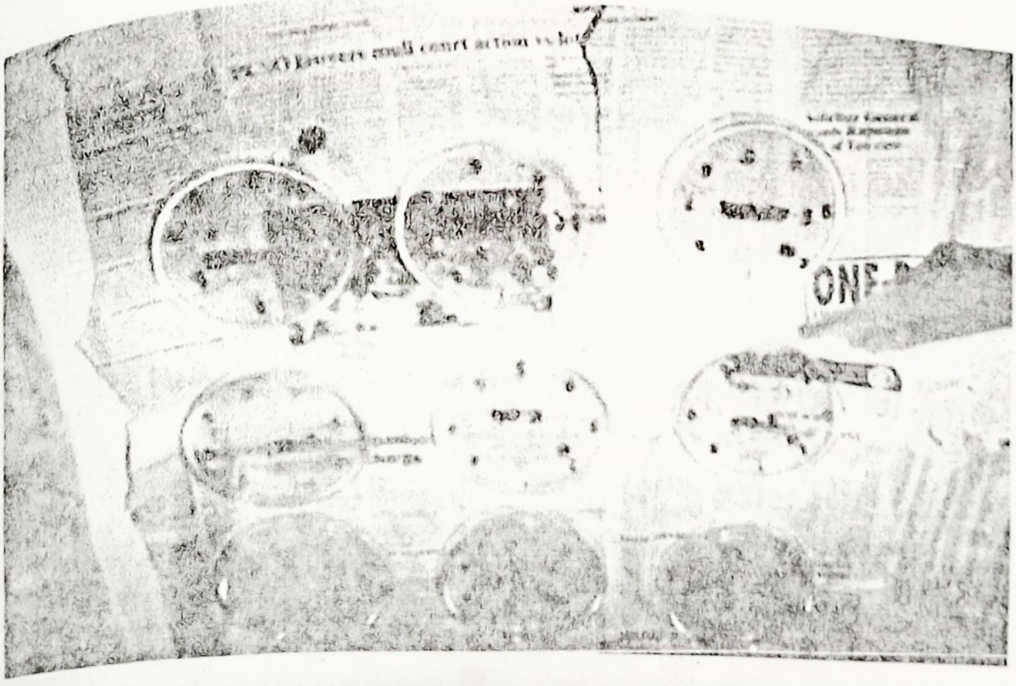


Plate No. 12. Diameter of zone inhibition are ready to be measured