

Marine actinomycetes from mangrove soil in lianga bay essay sample



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Mangrove swamps are forested intertidal ecosystems that occupy sediment-rich sheltered tropical coastal environments. By trapping and stabilizing fine sediments, mangroves control the quality of marine coastal waters. Aside from maintaining coastal food webs and populations of animals, mangroves have an important role in pollution control through their absorptive capacity for organic pollutants and nutrients, and they play an important role in storm protection and coastal stabilization.

In the Philippines, our mangroves remain to be one of the less explored environments in terms of microbial biodiversity. As a unique intertidal ecosystem, mangroves can be important sources of novel microorganisms and products. The Actinomycetes on the other hand, are well known secondary metabolite producers and hence of high pharmacological and commercial interest.

Isolation of Actinomycetes from SedimentMethicillin resistant *Staphylococcus aureus* (MRSA) frequently causes nosocomial infections, is often resistant to most of the antibiotics and is one of the greatest challenges for modern antimicrobial therapy, particularly since the emergence of *Staphylococcus aureus* (*S. aureus*) with intermediate susceptibility to glycopeptides. (Blanc et al.) In the Philippines, it has reported on the prevalence of oxacillin-resistance of *S. aureus* strains as 18%, 24%, 18%, and 18% in the years 1999 to 2002, respectively. 13-15In a 9-month study (August 2000-May 2001) of hospital-acquired *S. aureus*, reported an 11. 7% resistance rate for MRSA, (Atilano et al.). Methicillin-resistant *Staphylococcus aureus* (MRSA) was responsible for an estimated 94, 000 life-threatening infections and 18, 650 deaths in 2005, Based on their findings, they estimated that for every

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100, 000 people living in the U. S. there were 32 cases of invasive MRSA in 2005. An estimated 128 cases occurred for every 100, 000 people aged 65 and over, (Boyles &, 2007).

MRSA bacteria are usually spread through skin-to-skin contact with someone who has an MRSA infection or who is colonised by the bacteria. Colonised means bacteria are present on your body but do not cause any symptoms. The bacteria can also spread through contact with towels, sheets, clothes, dressings or other objects that have been used by a person who is infected by MRSA, (NHS, 2011). Because Methicillin Resistant Staphylococcus Aureus adapts so easily and because rapid deaths to the hospital it becomes challenging to the increasing number of antibiotics, new antibiotics are being developed and tested to help battle MRSA to help out patients and hospital to battle MRSA.

Thus, the present study aims to study to is to find Bioactive Secondary Metabolite from Marine Actinomycete isolates from Mangrove soil and partially characterized the Bioactive Secondary Metabolites using TLC & UV absorbance This research project aims to investigate the antibiotic production potential of mangrove microbial populations, with focus on Actinomycetes, in soil samples from Lianga Bay, Surigao del Sur, Philippines. It intends to find cultivable antibiotic-producing Actinomycetes against Methicillin Resistant Staphylococcus aureus (MRSA).

Objectives of the Study

The objective of the study is to find Bioactive Secondary Metabolite from Marine Actinomycete isolates from Mangrove soil and partially characterized

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the Bioactive Secondary Metabolites using TLC & UV absorbance Specifically it intends to:

1. Isolate Marine Actinomycetes from Mangrove soil;
 2. Isolate Marine Actinomycetes against MRSA using Agar plug Assay; 3. Conduct Liquid Assay using Cylinder Cup Assay;
 4. Partially characterized the Bioactive Secondary Metabolites using TLC & UV Absorbance Test
- Statement of the Problem

This study was conducted to investigate the presence of antimicrobial agents in the isolated Philippine Actinomycetes against MRSA. In addition, the study was carried out to isolate the bioactive compounds of the actinomycetes and evaluate its antagonistic efficacy against the growth of MRSA. Specifically, it aims to answer the following questions:

1. Do the selected Philippine actinomycetes and its isolates inhibit the growth of MRSA?
2. Is there a significant difference between the 15 selected Philippine actinomycetes against MRSA in term of zone of inhibition?
3. Are the selected Phillipine actinomycetes potential sources of antibacterial?

Hypotheses

Based on the forgoing research problems, the following hypotheses are formulated: 1. The isolates of the selected Philippine actinomycetes do not inhibit the growth of MRSA. 2. There is no significant difference between 15 selected Philippine Actinomycetes against MRSA in terms of the zone of inhibition. 3. The selected Philippine Actinomycetes are not potential sources of antibacterial.

SIGNIFICANCE OF THE STUDY

Antibiotic has a huge function to the health and life period of human beings. They can care for bacterial infections (antibiotics), and help the society from rapid infections there is no doubt that antibiotic has save many lives over the years. A strain of MRSA that causes bloodstream infections is five times more lethal than other strains and has shown to have some resistance to the potent antibiotic drug vancomycin used to treat MRSA, according to a Henry Ford Hospital study. The study found that 50 percent of the patients infected with the strain died within 30 days compared to 11 percent of patients infected with other MRSA strains. The average 30-day mortality rate for MRSA bloodstream infections ranges from 10 percent to 30 percent, (Science Daily, 2010).

Vancomycin and other pathogens which may kill MRSA are more expensive and finding new medicine for MRSA would be good enough since it will add this for some antibiotics which can help fight MRSA Strains. In this study, Actinomycetes and bacillus were isolated from the mangrove soil collected from five (5) mangrove areas of Lianga, Surigao del Sur and screened against known pathogens. This study is to test the antibacterial activity of Actinomycetes and Bacillus bacteria against Methicilline Resistant Staphylococcus Aureus. It is hoped to discover new compounds which could lead to the generation of a local-based, economical antibiotic.

CONCEPTUAL FRAMEWORK

INHIBIT THE GROWTH OF MRSA

MANGROVE

SOIL

MRSA

ACTINOMYCETES

ISOLATE

Figure 1: Semitic Diagram of the Conceptual Frame work

From the Mangrove soil that had been isolated the Marine Actinomycete will be assayed using MRSA in the process of Agar Plug and Cylinder Cup Assay and will be lead us characterized the Bioactive Bacterial Secondary Metabolites in which this is the new Resistance against MRSA. SCOPE AND LIMITATION

The study entitled Marine Actinomycetes from Mangrove Soil in Lianga Bay, Philippines: Potential Source of Antibiotic against Methicillin-Resistant *Staphylococcus Aureus* (MRSA) concerns on the screening of Actinomycetes and *Bacillus* isolates from mangrove areas in Lianga, Surigao del Sur as potential antibacterial sources against Methicilline Resistant *Staphylococcus Aureus* (MRSA).

Partial characterizations of the Bioactive Secondary Metabolites were also done using TLC with subsequent Autobiography. TLC bands were also viewed under UV. The study was conducted last April 18, 2012 at National Institute of Molecular Biology and Biotechnology (BIOTECH) at University of the Philippines, Los Baños, Laguna under the supervision of Irene A. Papa and Teofila O. Zulaybar, Microbiologists and University Researchers.
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Definition of Terms

MRSA is a type of staph bacteria that is resistant to certain antibiotics called beta-lactams. These antibiotics include methicillin and other more common antibiotics such as oxacillin, penicillin, and amoxicillin. In the community, most MRSA infections are skin infections.

ACTINOMYCETES bacteria is a rod-shaped or filamentous, gram-positive, aerobic bacteria. It is common in soils, essential to growth of many plants and source of much of original antibiotic production in pharmaceutical industry. ANTIBIOTICS is a medicine which kills one specific type of pathogenic micro-organism (germ). This specific micro-organism is bacteria. Antibiotics are only effective on and approved for use to treat bacteria or to prevent a bacterial infection.

Running Head: Marine Actinomycetes Potential Source of Antibiotic against MRSA

Marine Actinomycetes from Mangrove Soil in Lianga Bay, Philippines:
Potential Source of Antibiotic against Methicillin-Resistant Staphylococcus aureus (MRSA)

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Marine Actinomycetes from Mangrove Soil in Lianga Bay, Philippines: Potential Source of Antibiotic against Methicillin-Resistant Staphylococcus Aureus (MRSA)

Bacterial resistance was first observed in early 1940s. Currently, more than 70% of bacteria which caused hospital-acquired infections were resistant to at least one of the antibiotics used to treat them (Purdon &, 2007).

Resistances of bacteria to antibiotic continue. Antibiotic resistance occurs when they continue to evolve over the year which can affects the life span of the people or worst can lead to rapid death. The wildly development of bacteria resistance lead to adjustment of antibiotic that can battle opposed to microorganism. Source: Centers for Disease Control and Prevention

MRSA infection is a major public health problem. For example, a federal report issued October 2007 by the Centers for Disease Control and Prevention reported that there were more than 94, 000 serious MRSA infection related deaths in the United States in 2005. The same report said that nearly 19, 000 MRSA related deaths occurred, more than the number of AIDS related deaths in the same year (shown in the graph). This suggests that even in developed countries where advance medical techniques are used, MRSA infection still kill thousands of people. In another report, over 85 percent of the MRSA infections occurred in hospitalized patients in health care institutions. The disease seems to foster in the medical facilities where antibiotic use is evident and decontamination is always practiced. This further suggests that resistance to drugs is usually associated with exposure to drugs. Thus, fast and accurate detection of bacterial transmission is crucial to better control of healthcare-associated infection. After detection, <https://assignbuster.com/marine-actinomycetes-from-mangrove-soil-in-lianga-bay-essay-sample/>

fast acting and novel drug should be administered. The extraordinary ability of certain bacteria to develop resistance to antibiotics —which are otherwise useful in speeding recovery from some illnesses — has been a hot topic on the minds of doctors, hospital staff, reporters, and the general public for several years. It is also heralded as a textbook example of evolution in action (Purdon &, 2007). The purpose of this study is to source out novel antibacterial compound to be able to answer to the demands of antibiotic needs. It is to find new and stronger antibiotic from Actinomycetes which fight Methicilline Resistant Staphylococcus Aureus.

The following literatures were reviewed to support this concept.

Bacterial Diversity in Mangrove Soil

Actinomycetes are found in soil hence their presence in mangroves. Mangroves are rich in Biodiversity in as much as there is decaying vegetation that provides nutrients for microbes to grow. All kinds of microbes are found in mangroves not just bacterial community as what the results of the research funded by CHED Zonal Research Center for Region IV-UPLB Project on Isolation, Preservation and ID of Microorganisms with Derivative and antimicrobial Potential from Mangrove Ecosystem in Mindoro reported. Fungi, which include both yeasts and molds, are found together with bacteria. Bacterial population (10⁶-10⁹ colony forming units/CFU) was found to be higher in number compared to molds (10⁴-10⁷) and the yeasts (10³-10⁴) are in much lower population (Zulaybar, et. al., 2004). Studies conducted in various sites abroad reported that mangrove soil provides an exclusive ecological place for microorganisms to reproduce. This is possible because mangrove soil is rich in nutrient to support these bacterial

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communities. In 2012, a study of Bacterial Isolates of the Mangrove Swamp Soils in Cross River Estuary, South-East Nigeria, reported that the bacterial isolates in the mangrove swamp soils of Cross River estuary, South-East, Nigeria, indicate that *Streptomyces* sp. is predominant followed by *Bacillus*, *Micrococcus* and *Pseudomonas* species. Their presence in such soils indicates that they have ability to degrade organic materials.

The *Staphylococcus* sp. and *Streptococcus* sp. found in short mangrove and *Nypa* palm respectively were regarded as invaders and contaminants introduced by human activities into the mangrove swamp forest. Mangrove swamp soils constitute a biological entity and therefore require conservation measures that would maintain their productivity. (Akpan, Solomon &, 2012) A study Microbial Diversity of the rhizosphere soil of, *Avicennia marina* and *Avicennia officinalis* collected from mangrove the biodiversity of microorganisms viz., bacteria, fungi and actinomycetes were isolated enumerated and identified from the rhizosphere soil samples of, *Avicennia marina* and *Avicennia officinalis* from reserved forest of Pichavaram were collected and analysed for this microbial diversity. Five bacterial isolates. These isolates were subjected to various biochemical tests and identified bacterial namely *Pseudomonas fluorescens*, *Alcaligenes* sp., *Bacillus subtilis* and *Serratia marcescens*.

The characterized fungal and actinomycetes isolates are *Aspergillus niger*, *Trichoderma Viride*, *Aspergillus flavus* and *Nocardia* sp (Thiripurasundari et, al. , 2010) A study Microbial diversity and ecology of the Soda Lakes of East Africa he microbes are obligately alkaliphilic or alkali-tolerant and many appear to represent separate alkaliphilic lineages within recognized taxa, <https://assignbuster.com/marine-actinomycetes-from-mangrove-soil-in-lianga-bay-essay-sample/>

indicating they may have evolved separately within the alkaline environment. As evaporative concentration continues, chloride ions also dominate in solution. As a consequence, a quite different population of prokaryotes is present in the trona (sodium sesquicarbonate) beds and concentrated lagoon brines of hypersaline lakes (Magadi-Natron basin) compared with more dilute lakes elsewhere in the East African Rift Valley. (Jones, Grant &, 1999)

Actinomycetes as Antibiotic

Actinomycete bacteria is a rod-shaped or filamentous, gram-positive, aerobic bacteria. It is common in soils, essential to growth of many plants and source of much of original antibiotic production in pharmaceutical industry (Microsoft Encarta, 2006). Actinomycetes are a group of bacteria that live mainly in the soil. Clusters of actinomycetes form long, thin filaments in the soil, and have an important role in the environmental carbon cycle. A hardy group of bacteria, they are particularly adept at surviving harsh conditions and breaking down tough substances in the soil, returning their components back down to the most basic structures. (Durkee, Lindle & 2003)

Actinomycetes play a quite significant role in natural ecological system and they are also prolific producers of antibiotics, antitumor agents, enzymes, enzyme inhibitors and immunomodifiers which have been widely applied in industry, agriculture, forestry and pharmaceutical industry. The Actinomycetes population density is less common in marine sediments relative to terrestrial soils (Sahoo et. al, 2009). The actinomycetes are particularly important in areas where there is a lot of decaying plant matter, such as forests and grasslands. Without their ability to participate in the <https://assignbuster.com/marine-actinomycetes-from-mangrove-soil-in-lianga-bay-essay-sample/>

decay of plant and fungi, nutrients from these dead organisms would not be restored to the soil for living plants to absorb and use.

Activated by high pH levels, a change in the soil's nutrient levels can deactivate the recycling processes of these organisms and drastically impact the soil ecosystem. When the soil contains a low pH level, not only are actinomycetes inactive but other soil decomposers, fungi, is activated. With this shift, soil conditions change and can become favorable for a new set of plants, such as unwanted weeds and invasive species. (Durkee, Lindle & 2003) The diversity of actinomycetes was greatest in soil samples of a primeval forest, with an average of 9.0 genera isolated, and followed by secondary forest and vegetable farmland samples, with averages of 6.7 and 6.5 genera isolated, respectively. The upper limit for the occurrence of thermophilic actinomycetes is about 3,500 m above sea level in Yunnan. Psychrophilic actinomycetes were isolated at up to the same altitude. In addition, the drier and poorer the soil was and the cooler the climate was, the lower the count of actinomycetes was and the higher the percentage of streptomycetes observed was. The genus *Streptomyces* appears to be the most important in ecological function. It represents up to 90% of all soil actinomycete diversity in Yunnan and is likely an important characteristic of the soil actinomycete population. (Jiang et, al. 1996) Actinomycetes are abundant in soil, and are responsible for much of the digestion of resistant carbohydrates such as chitin and cellulose.

They are responsible for the pleasant odor of freshly turned soil. Many actinomycetes and other actinobacteria are well known as degraders of toxic materials and are used in bioremediation. They are particularly well adapted

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to survival in harsh environments. Some are able to grow at elevated temperatures ($> 50^{\circ}\text{C}$) and are essential to the composting process. Members of the genus *Actinomyces* are normal commensal members of human oral cavities. They can cause serious infections when they invade tissues through breaks in the oral mucosa. The disease is becoming less common, but is still present in the USA, especially in inner city populations. (Burge &, 1994) Secondary metabolism of microorganisms has been the major source of bioactive compounds, such as antibiotics and immunosuppressant. Among the vast array of microorganisms, gram-positive soil bacteria *Streptomyces* are regarded as the most versatile and most capable producers of bioactive compounds, as is evident from the fact that more than 70% of commercialized antibiotics have been found from this genus. MRSA

The experts are sounding the alarm about antibiotic resistance because of grim new evidence: Drug-resistant strains of *Staphylococcus aureus*, a bacterium linked to a wide range of human diseases, are present in meat and poultry from U. S. grocery stores at unexpectedly high rates, (Science Daily, 2011) Prisons, military barracks, and homeless shelters can be crowded and confined, and poor hygiene practices may proliferate, thus putting inhabitants at increased risk of contracting MRSA. (David, et. al., 2010). The rate of MRSA infections in hospital patients has increased in recent years, according to a new study. Results show that in 2003, an average of 21 out of every 1, 000 hospital patients developed an infection with the bacteria commonly called MRSA, or methicillin-resistant *Staphylococcus aureus*. In 2008, that number was 42 out of 1, 000 patients.

The study involved patients at nonprofit academic medical centers in the United States. MRSA is a strain of bacteria that's resistant to the antibiotics used to treat staph infections (FoxNews &, 2012) A population-based study of the incidence of MRSA infections in San Francisco during 2004-05 demonstrated that nearly 1 in 300 residents suffered from such an infection in the course of a year and that greater than 85% of these infections occurred outside of the healthcare setting (Liu C, et al., 2008)

CHAPTER II

METHODOLOGY

This section presents a discussion of the method, design and layout, the research variables, the subjects and sampling procedures, the research tools and instruments, and the statistical procedures utilized for the analysis and interpretation of the data. I. Materials:

Screen cap test tubes
Petri dishes (pyrex and disposable plates)
Pipettes

(25mL)
White tip

2 L-rods
30 Screw cap test tubes

5 dilution bottles
Starch

Yeast Extract
Malt Extract

Triple Beam Balance
Glucose/Dextrose

Nutrient Broth
Erlenmeyer flask (50mL)

45 Test Tubes
Pipettor (10mL)

Toothpicks
2 Alcohol lamps

Beakers (500mL)
Graduated cylinders (500mL)
Autoclave
Pipettes (10mL)

5 glass plates
Incubator

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Laboratory Oven Nutrient Agar

Distilled Water

Legend:

R - Replicates

X - Zone of Inhibition (ZOI, mm)

Using random sampling, soils samples were collected from five (5) mangrove sites. Bacteria were isolated using selective media. Isolated Marine Actinomycetes were screened against Methicilline Resistant Staphylococcus Aureus, using Agar Plug Assay and Cylinder Cup Assay. The Extract of Actinomycetes were screened against MRSA.

III. Procedure

A. Isolation of Actinomycetes and Bacillus

A. 1. Collection of Soil Sample

Fifteen (15) one thousand grams (1000g) soil samples was collected in Mangrove Areas in Wakat, Barobo, Surigao del Sur Ganayon, Lianga, Surigao del Sur Diatagon, Lianga, Surigao del Sur San Agustin, Surigao del Sur Marihatag, Surigao del Sur for isolation and was air dried and placed in clean containers labeled properly. The collection area was located in 5 locations along Lianga Bay. Five (5) collection areas were selected. 3 soil samples were collected per sample area.

A. 2. Isolation of Actinomycetes and Bacillus

The isolation of bacteria was done using the procedure used by the UP-

BIOTECH, Antibiotic Production Laboratory.

A. 2. 1. Preparation of Materials and Media

Forty (40) petri plates; white tips; sixty 90mL dilution blanks (0.85% NaCl in distilled water plus 1g CaCO₃ in screw-capped test tubes; Four 300mL YMA with 1.5% NaCl; and four 300mL modified water agar with 1.5% NaCl; L-rod and toothpicks were sterilized at 15psi in 121 degrees for 15 minutes. Sterile YMA was then plated using disposable petri plates and allowed to solidify at room temperature. Dried plates were used for plating bacterial spores using Spread Plate Technique.

A. 2. 2. Dilution of Bacterial Spores for Plating

Ten grams (10g) of soil samples was added to a solution of 90mL distilled water, 0.85% NaCl and 1gram CaCO₃ in 250mL in Erlenmeyer flask and shake vigorously. Using 10-fold dilution technique, each sample was diluted in dilution banks from 10⁻¹ to 10⁻⁶. Dilution 10⁻³ to 10⁻⁶ were plated into the YMA plates and incubated upside down at 30°C for 24hrs.

A. 2. 3. Plating Bacterial Spores to Agar Medium

A. 2. 3a. Inoculation of Dilution in Media

One milliliter (1mL) of dilutions 10⁻³, 10⁻⁴, 10⁻⁵, and 10⁻⁶ were plate in YMA Plates. The plates was properly labeled and incubated at 37 ° Celsius for 48 hours. After 48 hours, the plate was evaluated for growth of bacterial colonies and possible contaminations. Colonies has been counted and properly recorded to identify which bacterial isolated came from which soil sample. Spreader has also noted.

A. 3. Purifying the Bacterial Culture

A. 3. 2. Colony Selection and Streaking

Selecting colonies was done using sterile toothpicks. Each colony was transferred to YMA Plate in quadrants. The plates was then be incubated at 30° Celsius for 3 days.

A. 3. 3. Purifying Cultures

Cultures were streaked in YMA quadrants were checked every day for 3 days to assist contaminations. Purified cultures were then used to prepare agar plugs. B. Antibacterial Screening (Agar Plug Assay)

Agar Plug Assay was done.

B. 2. Making Agar Plugs

The 42 bacteria (42 actinomycetes) streak in agar was used for the assay. A sterile cork borer was used to bore an agar plug from the quadrants of purified culture of Actinomycetes. B. 3. Agar Plug Assay

A total of 42 Nutrient Agar Plates was used. Each plate contains agar plugs. All Petri dishes were numbered according to its capacity and enough space for each agar plug.

B. 3. 1. Preparing Plates and Inoculation of Test Organisms Ten (10) mL of Nutrient Agar was poured to each of the sterile Petri plates and allowed to solidify. 1mL of Modified Water Agar inoculates with the test an organism was poured to specifically label dish (0 plates for test organism). After the soft agar solidifies, Agar plugs from the quadrants was picked using sterile toothpicks and placed upside down to the plates following the specific labels for its position in the plates. The plates were incubated at 30° Celsius for 24 hours. B. 3. 2. Reading the Zone of Inhibitions

Using digital Vernier caliper, zone inhibition in (mm) was measured, recorded and tabulated for analysis. B. 3. 3. Waste Disposal

Petri plates were used in the screening and then will be autoclaved for 15 minutes at 15psi and 121 degrees Celsius before disposals of media seeded with pathogens. A disposable plate was placed in separate bins for disposal <https://assignbuster.com/marine-actinomycetes-from-mangrove-soil-in-lianga-bay-essay-sample/>

while other plates were washed with soap and water before storage. The materials and apparatuses which come in contact with the pathogens were subject for decontamination for 15 minutes at 15psi and 121 degrees Celsius before washed and stored. Disposables will be placed in the respective garbage bins.

C. Stocking Pure Cultures

C. 1. Cultures in Slants

Test tubes with 5mL NA was prepared for the bacillus slants and the isolated bacteria was inoculated using inoculating loop. A MHA slant has also prepared for actinomycetes isolates. The slants was incubated for 24 hours and stored in cold room waiting further testing.

D. Liquid Assay

Cylinder Cup Assay was done in UP-BIOTECH. The assay is done using cylinder cups was with MRSA growth. D. 1. Preparation of Media

three (3) petri plates; white tips; sixty 90mL dilution blanks (0. 85% NaCl in distilled water plus 1g CaCO₃ in screw-capped test tubes; Four 300mL YMA with 1. 5% NaCl; and four 300mL modified water agar with 1. 5% NaCl; L-rod and toothpicks were sterilized at 15psi in 121 degrees for 15 minutes. Sterile YMA was then plated using disposable petri plates and allowed to solidify at room temperature. Dried plates were used for plating bacterial spores using Spread Plate Technique. D. 2. Extraction using Ethyl Acetate and Centrifuge

The 2 bacteria isolate Extract NBB3 and YBA12 were cultured in CFM Medium and using Ethyl Acetate are concentrate to 10000 ppm using Rotary Evaporator. These extract was label number 14 and 15 respectively.

D. 3. Cylinder Cup Assay

A total of 3 MRSA Plates was used and assayed. All Petri dish were numbered according to its capacity and enough space for each to easily determine the

highest ZOI. E. Reading of Zone of Inhibitions

Using digital Vernier Caliper, zone of inhibition in (mm) was measured, recorded and tabulated for analysis.

E. 1 Waste Disposal

Petri plates used in the screening were subjected for autoclaved for 15 minutes at 15psi and 121 degrees Celsius before disposals of media seeded with pathogens. Disposable plates were placed in separate bins for disposal while other plates were washed with soap and water before storage. The materials and apparatuses which come in contact with the pathogens were subjected for autoclaved for 15 minutes at 15psi and 121 degrees Celsius before washed and stored. Disposables were placed in the respective garbage bins. F. Thin Layer Chromatography (TLC)

For the TLC, using capillary tube, extracts were spotted directly onto TLC plate (Silica Gel 60, Mercks). The TLC Plate was dipped in a solvent system using toluene: acetone: methanol in ratios: 7: 3: 1 (v/v/v) to separate the individual components. G. Visualization of the Chromatogram

To visualize the compounds, the TLC plate was viewed under ultraviolet light with short and long ultraviolet wavelength of 254 nm and 366 nm, respectively. Spots visualized, were encircled using pencil (the graphite from the pencil was not eluted). The chromatogram was recorded in terms of a number called the RF (rate of flow). The RF value is the characteristics of a

specific compound under specified conditions (absorbent and developing solvent and is computed as follows:

$RF = \frac{\text{Distance compound has traveled from origin}}{\text{Distance developing solvent has traveled from the origin}}$

The distance the compound has moved is measured from the center of the zone of the origin line. For irregular or elongated spots, measurement was done by estimating the location of the center. H. Bioautography

In Bioautography, The TLC plate was freed of the developing solvent and was then thinly applied with LB agar medium, seeded with the locally isolated clinical MRSA strains. The seeded TLC plate was then incubated overnight to allow the growth of MRSA. After incubation, MTT was sprayed onto the seeded plate. MTT is a colorless tetrazolium salt that can be converted to a purple-colored formazan when acted upon by the dehydrogenase enzyme of the living test organism. It is used as the redox stain in order to visualize the microbial growth and detect the inhibition zones. If an antimicrobial compound is present, the antimicrobial activity is detected as a colorless zone of inhibition on the agar surface surrounded by a purple background.

K. Decontamination and Disposal of Microbes

The Bacterial Cultures, and all laboratory glass wares, pipettes, treated Petri dishes which come in contact with the microorganisms tested were autoclaved at 121°C and 15psi for 15 minutes, the decontamination materials were allowed to cool and nutrient media were discarded in the designated trash bin. All materials were washed thoroughly with detergent and water while the work area was cleaned and disinfected with chlorox.

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