A STUDY ON THE ANTIMICROBIAL PROPERTIES OF ETHYL ACETATE EXTRACT OF *Bryophyllum pinnatum* FIBROUS STEM

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UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY.

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DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY

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BENIN CITY, NIGERIA.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this work was carried out by ORHUE, Ruth Osariemen as an undergraduate final year project work in a partial fulfillment of the requirements for the award of the Doctor of Pharmacy (Pharm. D) degree and submitted to the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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CERTIFICATION OF THESIS ON PLAGIARISM

We the undersigned attest and declare that the thesis of ORHUE, Ruth Osariemen titled: STUDIES ON THE ANTIMICROBIAL PROPERTIES OF ETHYL ACETATE EXTRACT OF *Bryophyllum Pinnatum* FIBROUS STEM has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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DEDICATION

This project work is dedicated to God for guiding me through the Faculty of Pharmacy, University of Benin up to this blessed day, Iam very grateful.

ACKNOWLEDGEMENT

I am most grateful to God who saw and kept me through the Faculty of Pharmacy.

To my supervisor Dr. (Mrs.) UPE Francisca Babaiwa, thankyou very much ma for your guidance every step of the way in this project, the constant supervision and kind corrections and also the academic knowledge you have invested in me. May God continue to bless you in all you do.

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ABSTRACT

The emergence of multi-drug resistant microbes has rekindled the interest on plant derived compounds as alternatives to existing antimicrobial agents.

This study determined the antimicrobial property of ethyl acetate extract of the fibrous stem of *Bryophyllum pinnatum* and phytochemical constituents of the fibrous stem. The ethyl acetate extract of the fibrous stem did not show any antimicrobial activity against the clinical fungal and bacterial isolates at 0.3g/mL concentration of the extract.

Phytochemical analysis showed that alkaloids, flavonoids, terpenoids, tannins,saponins and glycoside which are responsible for antimicrobial activity were not detected.

Gas Chromatography -Mass Spectroscopy also identified various phytocomponents. The ethyl acetate extract did not show anti-microbial activity.

KEYWORDS: *Bryophyllum pinnatum*, Ethyl acetate, Fibrous stem, Antimicrobial, Phytocomponents.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background of the Study

Microbial infections represent a significant and growing health challenge worldwide. Among these microorganims—caused by the overgrowth of some organisms that are part of the normal flora of the body, like, *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus* or caused by opportunistic organism like *Pseudomonas aeruginosa*, *Trichophyton rubrum, Trichophyton terrestre*, *Saprochaeta capitata* and *Aspergillus nidulans.* Microorganisms possess diverse enzymatic systems that enable them to colonize and invade host tissues. These enzymes, such as proteases, lipases, and glycosidases, facilitate the degradation of structural components within the host, thereby promoting infection and persistence. Collectively, microbial infections constitute a major public health concern worldwide, affecting millions of individuals across different age groups and socioeconomic settings (Bhatia & Sharma, 2020).

Although such infections are often non-life-threatening, they contribute significantly to morbidity, discomfort, and secondary complications, including chronic inflammation and tissue damage. The impact is particularly pronounced in tropical and subtropical regions, where warm and humid climatic conditions favor microbial proliferation, and inadequate access to healthcare and effective antimicrobial therapy further exacerbates the problem (Havlickova et.al., 2018).

Conventional antifungal drugs such as azoles, allylamines, and griseofulvin have been widely used to manage fungal infections. However, due to increasing resistance, drug toxicity, prolonged treatment regimens, and high recurrence rates of the infections highlight the limitations of current therapie (Monod & Mehul, 2019).Also, conventional antibiotics such as penicillins, cephalosporin, tetracyclines have been used to manage bacterial infections and met with the same problem. This situation necessitates the search for alternative antimicrobial agents, preferably derived from natural sources with lower toxicity and broader mechanisms of action.

Plants have historically served as rich sources of bioactive compounds with antimicrobial properties. *Bryophyllum Pinnatum* (synonym Kalanchoepinnata), a succulent plant of the family Crassulaceae, has been extensively used in ethnomedicine across Africa, Asia, and South America for the treatment of infections, wounds, inflammation, and other ailments (Akacha et al., 2016; Ramesh et al., 2021). Phytochemical investigations reveal that *B. pinnatum* contains flavonoids, alkaloids, tannins, terpenoids, and other secondary metabolites known for antimicrobial activity (Ogunmefun et al., 2020).

Ethyl acetate extracts of plant materials, including *B. pinnatum*, have attracted attention because the solvent selectively extracts medium-polarity phytochemicals such as flavonoids, terpenoids, and phenolics that possess antimicrobial properties (Ginting et al., 2019). This raises the potential of the ethyl acetate fraction of *B. pinnatum* fibrous stem extract as effective antimicrobial agents. Recent studies have also emphasized the importance of Gas Chromatography-Mass Spectrometry (GC-MS) analysis in identifying bioactive phytocompounds responsible for antifungal and antibacterial activity (Pavithra et al., 2022).

Thus, exploring the antimicrobial potential and phytochemical profile of the fibrous stem of *B. pinnatum* provides a scientific basis for validating its ethnomedicinal use, discovering novel antimicrobial agents, and addressing the pressing challenge of antimicrobial resistance. Bacterial and fungal infections remain highly prevalent, especially in developing countries due to the environmental, socioeconomic, and healthcare factors that promote their persistence.

The limitations of existing antibacterial and antifungal drugs—including resistance, high cost, limited availability, and undesirable side effects—necessitate alternative therapeutic strategies (Yin et al., 2019).

Despite the widespread use of *B. pinnatum* in folk medicine, scientific validation of its antimicrobial activity, particularly the ethyl acetate fraction of its fibrous stem, remains limited. Few studies have investigated its antimicrobial l potential using standardized antimicrobial susceptibility methods. Moreover, detailed phytochemical profiling via GC-MS of its stem extracts has not been extensively reported in the last decade. This knowledge gap hampers the integration of *B. pinnatum* into evidence-based antimicrobial drug discovery and ethnopharmacological validation.

1.2 Scientific classification of *BryophyllumPinnatum* (Lam.) Oken

Kingdom: Plantae

Division: Spermatophyta

Class: Magnoliopsida

Order: Saxifragales

Family: Crassulaceae

Genus: *Bryophyllum*

Species: *pinnatum*

Common name: Miracle leaf, Life plant, Maternity plant, Air plant

1.2.1 Description, Distribution and Ethnobotanical Uses of *B. pinnatum*

Bryophyllum Pinnatum (Lam.) Oken belongs to the Crassulaceae family. It is native to Madagascar but widely distributed across tropical Africa, Asia, and South America (Ramesh et al., 2021) found in warm, temperate climate and can grow up to 2 meters tall. The leaf which is the main organ of propagation is succulent to the touch and crenate in nature. The plant can also be propagated through the stem with its root hairs. The leaves of the plant are used locally in Nigeria to quicken the healing of the navel of a newborn baby and its falling of. The plant is also valued for its antimicrobial, anti-inflammatory, wound-healing, and analgesic properties. Different plant parts—including leaves, roots, and stems—are used in traditional medicine to treat cough, ulcers, kidney stones, and skin infections (Ogunmefun et al., 2020).

1.2.2 Phytochemical Constituent

Phytochemical analyses have identified flavonoids, alkaloids, triterpenoids, steroids, tannins, glycosides, and phenolic compounds in *B. pinnatum*. Stem and root extracts are particularly rich in triterpenoids and alkaloids that may contribute to antimicrobial activity (Prasad et al., 2019). These phytochemicals are also present in other parts of the plant though in varying concentrations. The various phytochemicals are discussed below;

1.2.3 Flavonoids

These are a large group of compounds found in plants, especially in vegetables, fruits, nuts, stems,flowers, leaves and seeds. They are phenolic in nature being either high-molecular weight or low-molecular weight. They possess a three ring structure for which they are known C6 – C3 – C6. (Asad and Sidra 2020)

1.2.4 Alkaloids

They are nitrogen-containing naturally occuring compounds that often contain the nitrogen in a ring structure (heterocyclic). These compounds are basic in nature and are capable of forming salts. They are found in the plants, virtually all parts of the plant, the leaves, roots, barks, fruits and seeds. Although the concentration, among these parts can vary across species.

1.2.5 Triterpenoids

These are a group of naturally occuring compounds derived from squalene. They are present in plants especially in the bark, root and leaves protecting against hungry herbivores. They could be saponins, steroids and phytosterols. They are also responsible for the bitterness found in some plants and also the foaming properties of some plants.

1.2.6 Steroids

Steroids in plants, also known asphytosterols, are a group of naturally occurring compounds that are essential for normal plant growth and function. They are derived from triterpenoids and possess overlapping structures. They are found in virtually every plant part like leaves, stems, barks, roots, seeds and fruits but in varying concentrations which could be due to environmental conditions.

1.2.7 Tannins

They are a group of compounds that are natural astringents and are bitter in nature. They are capable of binding to proteins and other molecules as they are polyphenolic. They are found in barks, leaves, wood, roots and unripe fruits. The astringent property of the compound give unripe fruits its taste. They are high in concentration in leaves and unripe fruits to discourage animals from eating them. They also possess antioxidants properties and are used in tanning leather.

1.2.8 Glycosides

These are compounds that possess in their structure a sugar molecule linked to another functional group as a result serving a variety of purposes from storing energy, aiding in detoxification and providing defense against herbivores as they are important constituents of many medicinal plants.

1.2.9 Phenolic Compounds

They are a diverse class of plant secondary metabolites necessary for plant defense, stress resistance, and coloration. They possess an aromatic ring with one or more hydroxyl groups and include major groups like flavonoids, phenolic acids, lignans, and tannins and also have significant antioxidant,antimicrobial, and anti-inflammatory properties providing beneficial effects in human health. They are found in fruits, vegetables, grains, and beverages like tea and wine.

1.3 Ethyl Acetate Extracts in Phytomedicine

Ethyl acetate is a medium-polarity solvent that efficiently extracts a wide range of bioactive compounds, especially flavonoids, phenolics, and terpenoids. Studies have shown that ethyl acetate extracts of medicinal plants often display strong antifungal, antibacterial, and antioxidant activities (Ginting et al., 2019).

1.3.1 Previous Studies on *Bryophyllum Pinnatum* Extracts (2017-Present)

Several studies have shown that *Bryophyllum Pinnatum* possesses broad antimicrobial activity against different species of bacteria and fungi. For example, Ogunmefun et al. (2020) reported that the methanolic leaf extract showed strong activity against Candida albicans. Similarly, Akinpelu et al. (2019) and Ezeigbo et al. (2017) found that extracts from the leaf and stem inhibited the growth of Staphylococcus aureus, *Escherichia coli* and *Pseudomonas aeruginosa*.

Akacha et al. (2016) also observed that the root extract exhibited both antibacterial and antifungal effects. Despite these findings, very few studies have specifically focused on the ethyl acetate fraction of the fibrous stem or root, especially in relation to its activity against dermatophytes and co-existing bacteria found in skin infections. This gap in literature provides the basis for further investigation into the antimicrobial potential of this part of the plant.

1.4 Studies on Ethyl Acetate Extracts of Other Medicinal Plants

Ethyl acetate is a semi-polar solvent that is known to extract important bioactive compounds such as flavonoids, phenols, terpenoids and alkaloids. Many researchers have reported good antimicrobial results using ethyl acetate extracts of other medicinal plants. For instance, extracts of *Azadirachta indica* (neem) and *Ocimum gratissimum* (scent leaf) have been shown to inhibit the growth of Trichophyton mentagrophytes, Candida albicans, *Staphylococcus aureus* and *Escherichia coli* (Singh et al., 2021; Okigbo et al., 2020).

These results suggest that the solvent can extract compounds with both antibacterial and antifungal properties. It also supports the use of ethyl acetate for preparing extracts from *B. pinnatum* for comparative testing and possible identification of its active constituents.

1.5 Overview of Bacterial, Dermatophytes and other Pathogenic Fungi

Bacteria are microscopic, single-celled organisms that belong to the domain Bacteria. They are prokaryotes, meaning they lack a true nucleus and membrane-bound organelles. Bacteria could be gram negative or gram positive according to the stain absorbed. Also, they exhibit different shapes which include, coccus (spherical or round), bacillus (rod shaped), spiral (curved or helical), filamentous (long, thread-like filaments) and pleomorphic (variable; change shape depending on condition). There are hundreds of genera of bacteria usually classified according to the stain retained and their shape. Others include Acid-fast bacteria, Rickettsia, Chlamydia and Mycoplasma.

Fungi eukaryotic, non-photosynthetic organisms that obtain nutrients by absorbing organic matter from their environment. They belong to the Kingdom Fungi, which includes molds, yeasts, and mushrooms.

Dermatophytes are keratinophilic fungi responsible for superficial infections in humans and animals. The three main genera include Trichophyton, Epidermophyton, and Microsporum. These organisms cause infections collectively termed tinea or ringworm (Gnat et al., 2020). Common infections include tinea capitis (scalp), tinea corporis (skin), and tineaunguium (nails).

In addition to dermatophytes, non-dermatophyte fungi such as *Candida albicans* and Malassezia species contribute to cutaneous mycoses. Opportunistic fungi like Aspergillus as well as *Saprochaeta capitata* also pose risks, particularly in immunocompromised patients. *Penicillium notatum* also contribute to cutaneous infection, onchomycosis, it also poses risk in immunocompromised patients but rarely.

1.6 Dose-Response Models in Antimicrobial Research

The dose–response model is important in assessing how effective a plant extract is against microorganisms. It shows the relationship between the concentration of the extract and the degree of inhibition or killing of the test organisms. The most common parameters used are the Minimum Inhibitory Concentration (MIC), which is the lowest concentration that stops visible growth, and the Minimum Bactericidalor Fungicidal Concentration (MBC/MFC), which represents the lowest concentration that kills the organism.

These values are determined using standard procedures such as broth microdilution, agar dilution or disc diffusion methods as described by the Clinical and Laboratory Standards Institute (CLSI, 2017). Mathematical models like the logistic or sigmoidal dose–response curve are sometimes applied to estimate potency and compare the extract with standard drugs. This approach helps to understand not just how active the extract is, but also how it compares in strength to known antimicrobial agents.

1.7 Comparative Studies Against Dermatophytes, Malassezia Species, and Bacteria

Different microorganisms do not respond equally to plant extracts. Studies have shown that while dermatophytes such as *Trichophyton rubrum,* Microsporum gypseum, and Epidermophyton floccosum are usually sensitive to plant-derived compounds, yeasts like Malassezia species may show variable sensitivity because of their lipid-rich cell walls and special metabolic needs (Leong et al., 2019).

Among bacteria, Gram-positive organisms like *Staphylococcus aureus* and Bacillus subtilis are generally more sensitive to plant extracts than Gram-negative species such as E. coli and *Pseudomonas aeruginosa*, mainly because Gram-negative bacteria have an additional outer membrane that restricts penetration of many compounds (Ghosh et al., 2022).

From these comparisons, it can be seen that *B.pinnatum* extracts should be tested not only against dermatophytes but also against bacteria and other fungi to fully understand their range of antimicrobial action and possible therapeutic usefulness.

1.8 Justification of Study

Although *B. pinnatum* is well-documented in ethnomedicine, few studies have rigorously evaluated the antimicrobial activity of its fibrous stem ethyl acetate extract against bacteria and fungi. Additionally, phytochemical profiling via GC-MS of the stem extract is scarce in recent literature. Addressing this gap will provide scientific validation and contribute to antimicrobial drug discovery

1.9 Aim and Objectives of the Study

Aim:

To investigate the antibacterial and antifungal potential and phytochemical profile (GC-MS) of the fibrous stem of *Bryophyllum pinnatum*.

Specific Objectives:

1. To prepare and concentrate the ethyl acetate extract of the fibrous stem of *B. pinnatum*.

2. To evaluate the antibacterial and antifungal activity of the extract against selected bacteria, dermatophytes and other fungi.

3. To determine the minimum inhibitory concentration (MIC) , minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the extract.

4. To analyze the phytochemical composition of the extract using GC-MS.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

(i) Reagents and chemicals

Ethyl acetate, Crystal violet, Lagols's Iodine and Immersion oil. Oxidase reagent, Kovac'sreagent, Urease reagent, Disinfectant (Dettol)(Reckitt Benkiser Nig. Ltd)

(ii) Culture media

Nutrient agar (Cheutenya Agro Biotech), Nutrient broth, Mueller hinton agar (Hi Flown Global Resources), Citrate agar, Sabouraud dextrose agar (Lifesave Biotech, USA).

(iii) Equipment

Hot air oven, Incubator, Autoclave, Compound light microscope, Refrigerator

(Thermocool, UK), Digital weighing scale, Rotary evaporator, GC-MS analysis Machine

model: 7890A GC system, 5675C Inert MSD with triple –Axis detector, Packing material:

Capillary column, Carrier gas: Helium gas , Flow rate: in the instrumentation parameter ,

Temperature programme:, Column length: 30m, Dimension: 30mx250umx 0.25um,

Column: Agilent 19091-433HP-5Ms 5%phenyl methyl silox

(iv) Glassware and other apparatus

Beakers, conical flasks, Universal bottles (MacCartney, Universal and Bijoux), measuring cylinders, glass stirrer, glass slides, Petri dishes and Pasteur pipette (All glasswares were products of Pyrex, England), Sterile syringes, Bunsen burner, cotton wool(SAM SOLO GLOBAL RESOURCES), sterile swab sticks, Surgical gloves, surgical blades, slide (Micropoint, China), Aluminium foil paper(PRC Manufacturers), micropipette (OEM Manufacturers) and wire loop.

(v) Clinical Isolates

Escherichia coli, Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacillus subtilis, Trichophyton rubrum, Trichophyton terrestre*, Aspergillus nidulans and Saprochaeta capitata.

2.2 Method

2.2.1 Plant Collection and Preparation

Fresh stems along with the root hairs of the plant (fibrous stem) were obtained from the junior staff quarters of the University of Benin, Edo State, Nigeria. The stems were washed twice to remove dirt and debris and allowed to surface dry on trampolines. The fibrous stems were then cut into tiny round and longitudinal pieces to allow for proper drying of the plant part. The fibrous stems were allowed to dry for 4 weeks at room temperature away from direct sunlight with regular stirring (every 24 hours), of the plant part to expose different sides of the plant part to the atmosphere. The cut plant part was regularly inspected for mould growth to avoid microbial contamination of plant to preserve potential antimicrobial phytochemicals.

After drying, the plant part was pulverized, powered and weighted at 979.14g. The powered plant part were exposed to the atmosphere for 1 hour on a plastic trampoline before being poured into an airtight container.

2.2.2 Extraction Procedure, Filtration and Concentration

The weighed powered plant part was then poured into two maceration jars of equal weighed plant parts and 1.5 litre of ethyl acetate was poured into each of them. The solvent was allowed to sip down into the plant to the bottom and stirred with a thick glass rod to allow for proper mixing. The plant part was allowed to macerate for 72 hours with regular stirring or shaking of the jar to ensure proper extraction of potential antimicrobial phytochemicals. After 72 hours, the plant part plus solvent was poured into a bowl, surgical gloves were worn and the plant parts were manually expressed of excess solvent and separated into another bowl. The remaining liquid in the bowl was filtered using the Whatman No. 1 filter paper.

The resultant liquid was concentrated and evaporated to dryness at the Department of Pharmaceutical Chemistry, University of Benin using a rotary evaporator. The extract was stored in well labelled glass vial at 4ºC before use. (Okokon,Udoh, Frank, Amazu, 2012).

2.3 Phytochemical Test

2g of the plant extract was weighed with the aid of a digital weighing scale, and then transferred into a clean dry smaller sample bottle and taken to the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin for phytochemical assay. The sample was placed in a beaker and dissolved in 40ml of ethyl acetate and filtered and the filtrate obtained. A test tube rack bearing clean dry test tubes was provided. Tests for alkaloids, carbohydrates, reducing sugar, saponins, tannins, terpenoids, phenolic compounds, proteins, flavonoids and lastly deoxysugars were all carried out using the filtrate.

2.3.1 General Tests for Alkaloids

Approximately 2mL of the filtrate was measured into the test tube and 2 drops of dragendoff's reagent was added to it and the formation of a dull coloured crystalline precipitate was recorded as a positive result for the presence of alkaloids. The same procedure was repeated for the other general tests for alkaloids; Wagner's, Hager's and Mayer's. For Wagner's, the formation of a darker shade of the filtrate was recorded as a positive result but for Hager's and Mayer's, the formation of a bubble at the bottom of the test tube and colour of the filtrate being retained were recorded as negative results respectively.

2.3.2 Tests for Carbohydrates

Molisch's Test

Approximately 2mL of the filtrate was measured into a test tube and 2 drops of 1% of alpha-naphtholand 2mL of sulphuric acid was added to it while the test tube was slanted. The formation of a green ring was observed at the interface between the filtrate and alpha-naphthol was recorded as anegative result.

Fehling's Test

Approximately 2mL of the filtrate was measured into the test tube and 2 drops of Benedict's solution A and B was added to the test tube respectively and boiled for 3 minutes. The observation of an orange precipitate formed after 3 minutes of boiling indicated a negative result.

2.3.3 Tests for Saponins

Frothing Test

Approximately 1mL of the filtrate was measured into a test tube and 10mL of sterile distilled water was added to it and shaken vigorously for 1 minute. The formation of bubbles less than 1cm after on standing for 15 minutes indicated a negative result.

Lieberman Burchard's Test for Steroidal Saponins or Phytosterols

A mixture of 1mL of chloroform and acetic acid anhydride was added to 2mL of filtrate and 2mL of concentrated sulphuric acid was added to it. The formation of a dark green colour (intense) observed indicated a positive result.

Fehling's Test for Saponins

Approximately 10mL of filtrate was measured into a test tube and 5mL of dilute sulphuric acid was added to it and boiled for 15 minutes and cooled. Then 2.5mL of the filtrate was measured into another test tube and 20%NaOH was added to it and boiled with 0.1mL each of Fehling's solution A and B for 2 minutes. The retention of the blue colour of Fehling's solution A and the green layer of the filtrate on top indicates a positive result.

2.3.4 Test for Tannins

Gelatin Test

Approximately 2mL of the filtrate was measured into a test tube and 2mL of 1% gelatin solution in 10%NaCl was added to it. The separation of the mixture into two layers with the top being green and the lower part translucent indicated a negative result.

2.3.5 Test for Terpenoids

Salkowski's Test

2mL of the filtrate was measured into a test tube and 2mL of chloroform was added to it and drops of concentrated sulphuric acid was added to it. The colour of the filtrate got darker (dark green) indicated a negative result.

2.3.6 Test for Phenolic Compounds

Ferric Chloride Test

Approximately 2mL of the filtrate was measured into a test tube and 5mL of distilled water was added to it, 2 drops of 5% ferric chloride was also added. An intense colouration was formed (very dark green) and recorded as a positive result.

2.3.7 Test for Flavonoids

Alkaline Reagent Test

Approximately 2mL of the filtrate was measured into a test tube and few drops of 20%NaOH was added to it and also few drops of dilute HCl was added. The observation of two separate layers with the upper layer being dark green in colour and the lower part being translucent indicated a negative result.

2.3.8 Test for Proteins

Xanthoproteic Test

Few drops of concentrated nitric acid was added to 2mL of filtrate. The turning of the solution to an orange colour indicated a positive result.

2.3.9 Keller Killiani's Test for Deoxysugars

Few drops of dilute acetic acid was added to 2mL of filtrate and 5% ferric chloride was added to it. Then to the surface, concentrated sulphuric acid was added to it. The formation of a brown ring at the interface indicated a positive result.

2.4 GC-MS analysis

1g of the plant extract was weighed into a clean dry sample bottle and sent for analysis. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using an Agilent USA system hyphenated to a 5975C mass spectrophotometer equipped with a triple-axis detector and an auto-injector fitted with a 10 µL syringe. Helium gas served as the carrier gas throughout the analysis. The separation was achieved on a capillary column (30 m in length, 0.2 µm internal diameter, and 250 µm film thickness) coated with phenyl methyl siloxane.

The operating conditions were as follows: ion source temperature (EI) was maintained at 250 °C, while the interface temperature was set at 300 °C. The column pressure was 16.2 psia with an out time of 1.8 min. A 1 µL sample was injected in split mode using a split ratio of 1:50, and the injector temperature was maintained at 300 °C. The column temperature program began at 35 °C (held for 5 min), then increased to 150 °C at a rate of 4 °C per minute, followed by a further rise to 250 °C at 20 °C per minute, and held for 5 min. The total run time was approximately 47.5 minutes.

Data acquisition and system control were performed using the MS Solution software provided by Agilent. The chemical compounds present in the extract were identified by comparing their mass spectra with standard spectra from the National Institute of Standards and Technology (NIST II) library database.

2.5 Antimicrobial assay

2.5.1 Specimen collection

The bacteria used for this study were obtained from stock cultures of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria. They are *Escherichia coli*, Staphylococcus aureus, *Pseudomonas aeruginosa* and Bacillus subtilis.

The fungi especially the dermatophyte used for this study were gotten from the heads of infected pupils of a local public primary school; Ekosodin primary school and a local secondary school, University of Benin Demonstration Staff School. The heads of infected pupils were gently scrapped and swabbed with sterile swab sticks moistened with normal saline. The swab sticks were placed in their containers and sealed with a masking tape.

2.5.2 Preparation and Identification of Test Microorganisms

The collected samples of the fungi were brought to the Pharmaceutical Microbiology and Biotechnology laboratory of the University of Benin and cultured in a Sabouraud dextrose broth after which it was subcultured onto a Sabouraud dextrose agar plate. Colonial growths were observed and further subcultures were done to isolate the fungal pure species. The macroscopy and microscopy of the fungal pure species were observed (Shalayetal., 2016; Larone 2018), gram staining was done on the fungi species to aid visualization (Knoll 2023). The fungi species were identified using an atlas (de Hooge et al., 2019).

Gram staining techniques and biochemical tests were also done on the bacteria species obtained from the Department of Microbiology, Faculty of Pharmacy, University of Benin, Edo State, Nigeria.

2.5.3 Preparation of stock solution

Ciprofloxacin stock solution of 0.5mg/mL was prepared in 10% DMSO solution. Fluconazole of 0.001g/mL was used as stock solution. Concentration of 0.3g/mL stock solution of the fibrous stem extract was prepared by dissolving 1g of extract in 3mL of sterile distilled water.

2.5.4 Antimicrobial susceptibility tests

Antimicrobial susceptibility test was carried out using agar well diffusion method (Murray et al., 2009) with some modifications. Mueller Hinton Agar and Sabouraud dextrose Agar were prepared and sterilized at 121ºC for 15 minutes using an autoclave. The prepared agars were allowed to cool and 30mL of each agar was dispensed into sterile petri dishes and allowed to set. Upon setting, the agar plates was dried to remove excess moisture using an hot air oven at 50ºC for 10 minutes. After drying the plates, the standardized organisms were prepared by suspending the organisms in 1ml normal saline or sterile water. The entire surface of the agar was seeded with the organisms. A sterile cork borer 10mm was used to bore two wells in each agar plate and the base of the wells were sealed with Mueller Hinton agar for the bacteria plates and Sabouraud dextrose agar for the fungi plates. Into one of the sealed wells 0.2mL ethyl acetate extracts of *B. pinnatum* fibrous stem was introduced using a calibrated micropipette with a rubber teat. The same procedure was carried out with ciprofloxacin 0.5g/mL and fluconazole 0.001g/mL were 0.2mL was emptied into the other well to give 0.1g and 0.0002g per well respectively. Negative control (without the extract and test standard drugs) and positive control (viability test for organisms used) were carried out for each set of experiment. The plates were allowed to stand for 30 minutes and were all incubated in an upright position for 18-24 hours for bacteria at 37ºC and at 25-28ºC for 48 hours for the fungi. The inhibitory zone diameters (IZD) were measured in millimeters (mm) using a ruler and a pair of dividers as an index of the killing or inhibitory action of the test agent against a given organism. All experiments were carried out under aseptic condition. The results were recorded.

CHAPTER THREE

RESULTS

3.1 Percentage Yield of Extract

The percentage yield of the ethyl acetate extract of *Bryophyllum pinnatum* fibrous stem (1.38%) with its consistency (dull green solid after complete removal of the extractive solvent is shown in Table 1.

3.2 Qualitative Phytochemical Investigation

The ethyl acetate extract of the fibrous stem of *B. pinnatum* revealed the presence of alkaloids, phenolic compounds, proteins, deoxysugars, and phytosterols. Carbohydrates, saponins, tannins, terpenoids, flavonoids were not detected as shown in Table 2.

3.3 Susceptibility Profile of Test Microorganisms to Ethyl acetate fibrous stem extract of *B. pinnatum* and analytical antimicrobial agents

The preliminary antimicrobials susceptibility profile of the fibrous stem of *Bryophyllum pinnatum* extract did not show any activity against the clinical isolates used which include; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Trichophyton rubrum, Trichophyton rubrum, Aspergillus nidulans* and *Saprochaeta capitata;* all at 0.3g/mL concentration of the extract as shown in Table 3

All the clinical bacterial isolates used showed susceptibility to Ciprpfloxacin with ranges from 20.00-52.50mm in IZD at 0.5g/mL concentration; while the clinical fungal isolates, only *Saprochaeta capitata* showed susceptibility to fluconazole with IZD, 30.00mm at 0.001g/mL concentration.

3.4 Compounds Identified in Ethyl acetate Extract of *Bryophyllum Pinnatum* Fibrous Stem Using Gas Chromatography - Mass Spectroscopy (GC-MS)

Twenty nine compounds were detected in *B. pinnatum* fibrous stem ethyl acetate extract, with; 1-Octadecene, Bromoacetic acid tetradecylester, 1-Heneicosanol,Bis(2-ethylhexyl) phthalate, Oleic acid, Bicyclo[3.1.1] heptane, 2,6,6-trimethyl, Farnesol isomer a, 2,4-Di-tert- butylpheno, 3-Eicosene, (E)-, 9, 12-Octadecadienoic acid (Z, Z)- and n-Hexadecanoic acid as the major peaks including other minor peaks. Further identification information, including Retention time, Area% greatly increases the reliability of this analysis and is reported in Table 4.

3.5 Bioactivity and Nature of Phytocomponents Identified in Ethyl acetate Extract of *B. pinnatum* Fibrous Stem by GC-MS

This shows the bioactivities of the various phytocomponents identified with 24 compounds having bioactivity as shown in Table 5.

Table 1 : Physical Properties and Yield of the *B. pinnatum* Fibrous Stem Ethyl acetate Extract

Solvent	Ethyl acetate
EXTRACT TEXTURE	Rough Powdery
COLOUR	Dull Green
CONSISTENCY	Dry, Cracked
ODOUR	Characteristic
PERCENTAGE YIELD	1.38%

Percentage Yield = (Weight of dry extract/Weight of dry plant) * 100%

Weight of dry extract = 13.6g
Weight of dry plant = 979.14g

Percentage Yield = (13.6/979.14) * 100%
Percentage Yield = 1.38%

Table 2 : Preliminary Qualitative Phytochemical Analysis of the fibrous stem of *Bryophyllum Pinnatum* Ethyl acetate Extract

Parameter	Occurrence
Alkaloids	+
Carbohydrates	ND
Saponins	ND
Phytosterols	+
Tannins	ND
Terpenoids	ND
Phenolic Compounds	+
Flavonoids	ND
Proteins	+
Deoxysugars	+

+: Present   ND: Not Detected

Table 3 : Susceptibility Profile of Test Microorganisms to Ethyl acetate Fibrous Stem Extract of *B. pinnatum* and Analytical Antimicrobial Agents

Microorganisms	Ethyl Acetate Extract (mm)	Ciprofloxacin (mm)	Fluconazole (mm)
Escherichia coli	NIZ	20.00	NA
Bacillus subtilis	NIZ	32.00	NA
Staphylococcus aureus	NIZ	43.50	NA
Pseudomonas aeruginosa	NIZ	52.50	NA
Trichophyton rubrum	NIZ	NA	NIZ
Trichophyton terrestre	NIZ	NA	NIZ
Aspergillus nidulans	NIZ	NA	NIZ
Saprochaeta capitata	NIZ	NA	30.00

NIZ - No Inhibition Zone; NA - Not Applicable

Table 4: Phytocomponents Identified in Ethyl Acetate Extract of *B. pinnatum* Fibrous Stem by GC-MS

Peak No.	Compound Name	Retention Time (min)	Area (%)
1	5-Tetradecene	5.696	1.02
2	4-Tetradecene	8.054	1.16
3	2,4-Di-tert-butylphenol	9.461	6.73
4	Bromoacetic acid	10.148	3.20
5	Undecanoic acid	11.916	0.70
6	1-Octadecene	12.019	3.06
7	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	12.425	5.19
8	9-Cycloheptadecen-1-one	12.517	0.60
9	6-Octen-1-ol, 3,7-dimethyl-, propanoate	12.637	1.15
10	9-Nonadecyne	12.797	2.35
11	Hexadecanoic acid	13.192	2.87
12	n-Hexadecanoic acid	13.553	23.95
13	1-Octadecene	13.701	7.77
14	Heptadecanoic acid	14.348	0.66
15	Methyl 5,12-octadecadienoate	14.548	2.29
16	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14.686	0.92
17	Nonanoic acid, 9-hydroxy-, methyl ester	14.766	0.55
18	9,12-Octadecadienoic acid	14.926	10.22
19	Linoleic acid ethyl ester	15.052	2.38
20	Oleic acid	15.092	4.60
21	1-Heneicosanol	15.246	3.27
22	Cyclopropaneoctanal, 2-octyl-	15.378	0.62
23	Farnesol isomer a	16.030	5.42

24	Benzeneacetaldehyde, α-(methoxymethylene)-4-nitro-	16.362	0.56
25	Hexacosyltrifluoroacetate	16.980	0.99
26	4-Dehydroxy-N-(4,5-methylene-dioxy-2-nitrobenzylidene) tyramine	18.691	0.57
27	Bis(2-ethylhexyl) phthalate	19.046	3.69
28	δ-Tocopherol	20.419	1.50
29	δ-Tocopherol	20.465	2.02
	Total Identified: 87		

Table 5 : Bioactivity and Nature of Phytocomponents Identified in Ethyl acetate Extract of *B. pinnatum* Fibrous Stem by GC-MS

Compound Name	Compound Nature	Bioactivity
2,4-Di-tert-butylphenol	Substituted phenol	Antioxidant, antibacterial, and antifungal (Versha et al., 2015; Zhao et al., 2020)
Phenol, 3,5-bis(1,1-dimethylethyl)	Substituted phenol	Antioxidant, anti-inflammatory (Rouvier et al., 2024; Tabassum et al., 2022)
Phenol, 2,5-bis(1,1-dimethylethyl)	Substituted phenol	Antioxidant, anti-inflammatory (El Shahir et al., 2022; Alabi et al., 2025)
Undecanoic acid	Saturated fatty acid	Antifungal, preservative (Rossi et al., 2021)
Dodecanoic acid	Saturated fatty acid	Antibacterial, antiviral, anti-inflammatory (Ameena, 2024; Huang et al., 2014)
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	Monoterpene	Antimicrobial, analgesic, anti-inflammatory, bronchodilator (Zaichikova et al., 2020; Zielinska-Blaja et al., 2020)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene alcohol	Chlorophyll constituent; antioxidant, analgesic, anticancer, anti-inflammatory (Santos et al., 2025)
Formic acid, decyl ester	Fatty acid ester	Antimicrobial, signaling molecule (Rokotofina et al., 2024)
n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	Antioxidant, lubricant, signaling molecule (Purushothaman et al., 2025)

Compound Name	Compound Nature	Bioactivity
Heptadecanoic acid (Margaric acid)	Saturated fatty acid	Antimicrobial (Lei et al., 2024)
Tridecanoic acid	Saturated fatty acid	Antimicrobial (Lei et al., 2024)
9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	Polyunsaturated fatty acid	Anti-inflammatory (Wang et al., 2024)
9,12-Octadecadienoic acid (Z,Z), methyl ester	Fatty acid methyl ester	Antimicrobial (Wang et al., 2024)
Linoelaidic acid (trans-fat)	Polyunsaturated fatty acid	Anti-inflammatory (Wang et al., 2024)
7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-	Monoterpene epoxide	Antimicrobial, antioxidant, anti-inflammatory, expectorant (Zaichikova et al., 2020; Zielinska-Blaja et al., 2020)
Linoleic acid ethyl ester	Fatty acid ester	Antimicrobial, emollient (Santa Maria et al., 2023; Pegoraro et al., 2021)
Oleic acid	Monounsaturated fatty acid	Antioxidant, anti-inflammatory (Santa Maria et al., 2023; Pegoraro et al., 2021)
Farnesol isomer a	Sesquiterpene alcohol	Antimicrobial (especially antifungal), antioxidant, anticancer (Decanis et al., 2021; Li et al., 2023)

Compound Name	Compound Nature	Bioactivity
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	Sesquiterpene alcohol	Antimicrobial (especially antifungal), antioxidant, anticancer (Decanis et al., 2021; Li et al., 2023)
2-Pyridinamine, N-(4,5-dihydro-5-methyl-2-thiazolyl)-3-methyl	Heterocyclic amine	Anti-inflammatory, analgesic, antimicrobial (Qadir et al., 2022)
Bis(2-ethylhexyl) phthalate	Phthalate ester	Endocrine disrupting chemical (Latini et al., 2005)
Diisooctyl phthalate	Phthalate ester	Endocrine disruptor (Latini et al., 2005)
δ-Tocopherol	Tocochromanol	Antioxidant, anticancer, anti-inflammatory (Brigelius et al., 1999)
7-Oxabicyclo[4.1.0]heptane-1-(2,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, [E]-	Monoterpene epoxide derivative	Antimicrobial, insecticidal, anti-inflammatory, aromatic/flavourant (Zaichikova et al., 2020; Zielinska-Blaja et al., 2020)

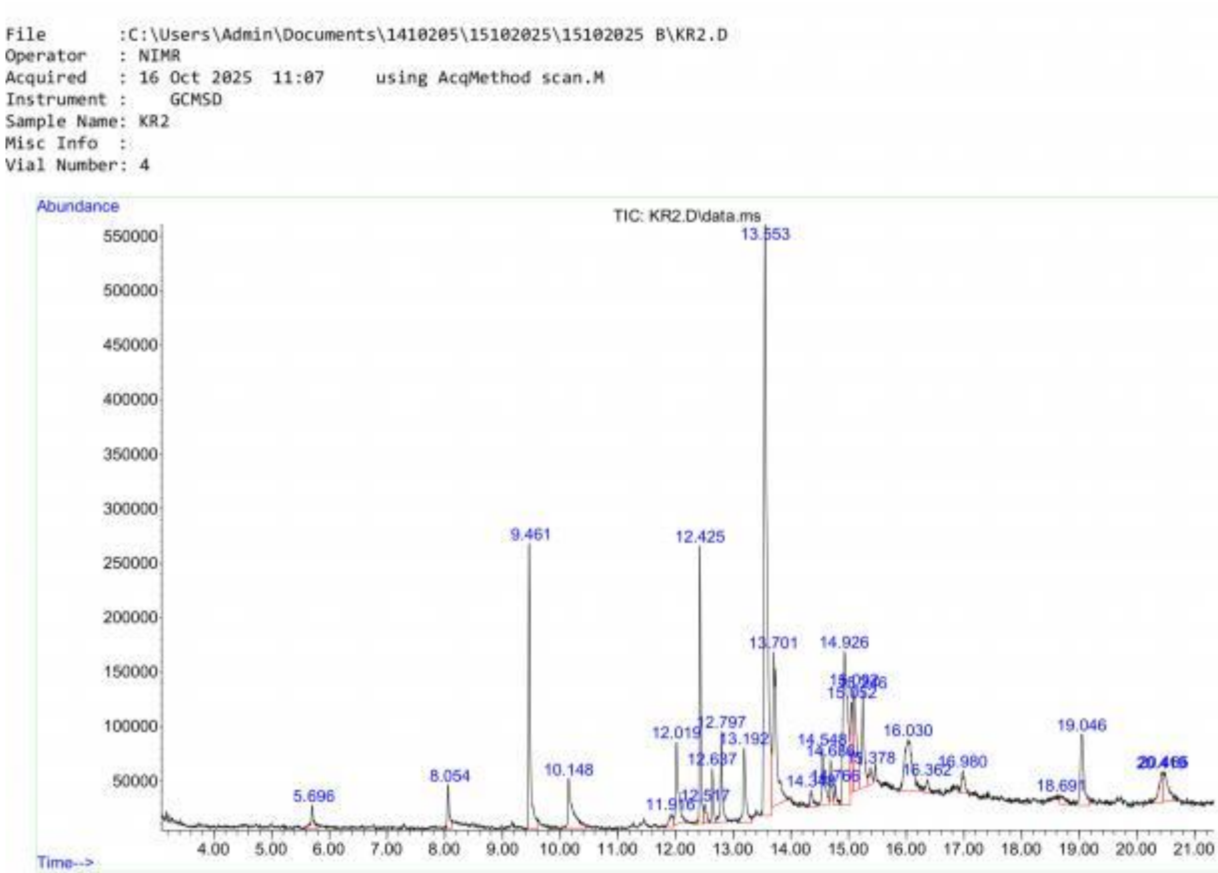

Figure 1: GC-MS Spectra of *Bryophyllumpinnatum* Fibrous Stem

CHAPTER FOUR

DISCUSSION

The extraction of the fibrous stem of *Bryophyllum pinnatum* using ethyl acetate gave only a small yield (1.38%) compared to the total weight (979.14g) of the plant material. This low yield suggests that ethyl acetate may have only extracted a small portion of the compounds present in the plant. It is also likely that the volume of solvent used (3L) was not enough to completely extract all the active constituents from the sample. The phytochemical screening showed the presence of alkaloids, phenols, phytosterols, proteins, and deoxysugars, while tannins, saponins, flavonoids, and carbohydrates were absent. The presence of deoxysugars indicates that some glycosidic compounds maybe present, which can affect how the active components dissolve, absorb, or show activity. The GC–MS analysis also confirmed the presence of substituted phenols, fatty acid esters, monoterpenes, diterpenes, and sesquiterpene alcohols. These results agree with the phytochemical screening and show that the extract mainly contained medium-polarity compounds instead of large polar ones like tannins or saponins (Chaithra et al., 2020).

When the extract was tested for antimicrobial activity using the agar-well diffusion method, no zone of inhibition was observed against any of the test bacteria or fungi. The microorganisms tested included *Escherichia coli*, *Pseudomonas aeruginosa*, Staphylococcus aureus, *Bacillus subtilis, Trichophyton rubrum, Trichophyton terrestre*, Saprochaeta capitata, and *Aspergillus nidulans.* This result shows that the ethyl acetate extract, at the concentration used, was not able to inhibit the growth of the organisms. Eventhough some of the detected phytochemicals, such as phenols and terpenes, are known to have antimicrobial properties, their amount in the crude extract might have been too low to produce an observable effect.

There area few possible reasons for this outcome. Firstly, ethyl acetate mainly extracts compounds of medium polarity, which means that highly polar antimicrobial substances such as saponins, glycosides, and flavonoids may not have been extracted. These compounds have been reported in other studies to contribute to the antimicrobial activity of*B.pinnatum* (Faloyeetal., 2025; Chaithra et al., 2020). Secondly, the solvent volume and extraction process used may not have been enough to obtain a concentrated or exhaustive extract. In addition, the microorganisms used in the study, especially Gram-negative bacteria and dermatophytes, have thick and complex cell walls that make them more resistant to many plant extracts. They may require a higher concentration or a combination of active compounds to show visible inhibition.

Although no antimicrobial activity was observed, the detected compounds are still significant. Phenolic compounds have been reported to possess strong antioxidant and antimicrobial effects (Obioma et al., 2017). Fatty acids and their esters can damage microbial cell membranes, making them more permeable (Agoramoorthy et al., 2007). Also, sesquiterpene alcohols such as farnesol and its analogues are known to interfere with fungal growth and development (Li et al., 2022). However, these compounds might have been present in small amounts or may not have diffused properly through the agar medium during testing. In the case of glycosides, they may require hydrolysis to release the active aglycones before showing any activity, and this was not done in this experiment.

In summary, the ethyl acetate extract of the fibrous stem of *Bryophyllumpinnatum* was found to contain several classes of bioactive compounds, but it did not show any antimicrobial or antifungal activity at the concentrations tested. This could mean that the active components of the plant are present in other solvent fractions, especially more polar ones, or that higher extract concentrations are needed. Future studies should consider using solvents like methanol or aqueous-alcohol mixtures, larger solvent volumes, and repeated extractions to improve yield. Broth microdilution methods can also be used to check for weaker or concentration-dependent antimicrobial effects that may not be detected by the agar diffusion method.

CHAPTER FIVE

CONCLUSION

From this research, it can be concluded that the fibrous stem of *Bryophyllum pinnatum* contains several important chemical groups, even though the ethyl acetate extract did not show any antimicrobial or antifungal activity in this study. The phytochemical and GC–MS analyses confirmed the presence of compounds such as alkaloids, phenols, phytosterols, fatty acid esters, and terpenes, which are known to have biological importance. However, their presence alone was not enough to produce an observable effect against the microorganisms tested. This could mean that the amount of these active substances in the ethyl acetate fraction was too low, or that the solvent was not the best choice for extracting the most active components of the plant.

The lack of inhibition against both bacteria and fungi also suggests that the extraction process and concentration used might not have been strong enough to demonstrate any activity. Since ethyl acetate mostly extracts compounds of medium polarity, some of the more potent antimicrobial agents, such as saponinsor flavonoids, which are usually more polar, may not have been extracted. It is also possible that the microorganisms tested, especially the dermatophytes and Gram-negative bacteria, were too resistant for the crude extract to have an effect at that concentration.

Overall, this study provides useful insight into the chemical composition of the fibrous stem of *Bryophyllum pinnatum* and highlights that while it contains compounds of potential pharmacological interest, its ethyl acetate extract did not show activity under the tested conditions. Future studies should consider using solvents of different polarities, larger extraction volumes, and more sensitive testing methods to get a clearer understanding of the plant's antimicrobial potential. Further purification or fractionation of the extract may also help in identifying and isolating the actual compounds responsible for any biological effects.

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APPENDIX

APPENDIX 1

Table 1: Antimicrobial Activity of Distilled Water

Organism	Inhibition Zone Diameter (mm)
Escherichia coli	0.0
Staphylococcus aureus	0.0
Pseudomonas aeruginosa	0.0
Bacillus subtilis	0.0
Trichophyton rubrum	0.0
Trichophyton terrestre	0.0
Aspergillus nidulans	0.0
Saprochaeta capitata	0.0

Table 2: Negative Control Observation of Clinical Bacteria Isolate

Organism	Inference
Escherichia coli	+
Staphylococcus aureus	+
Pseudomonas aeruginosa	+
Bacillus subtilis	+

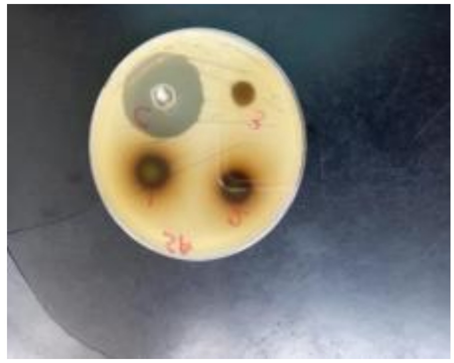
+: Growth

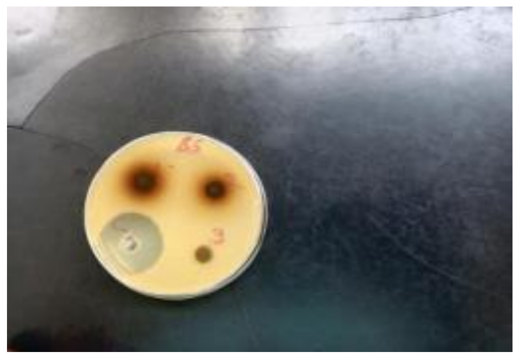
Table 3: Negative Control Observation of Clinical Fungal Isolates

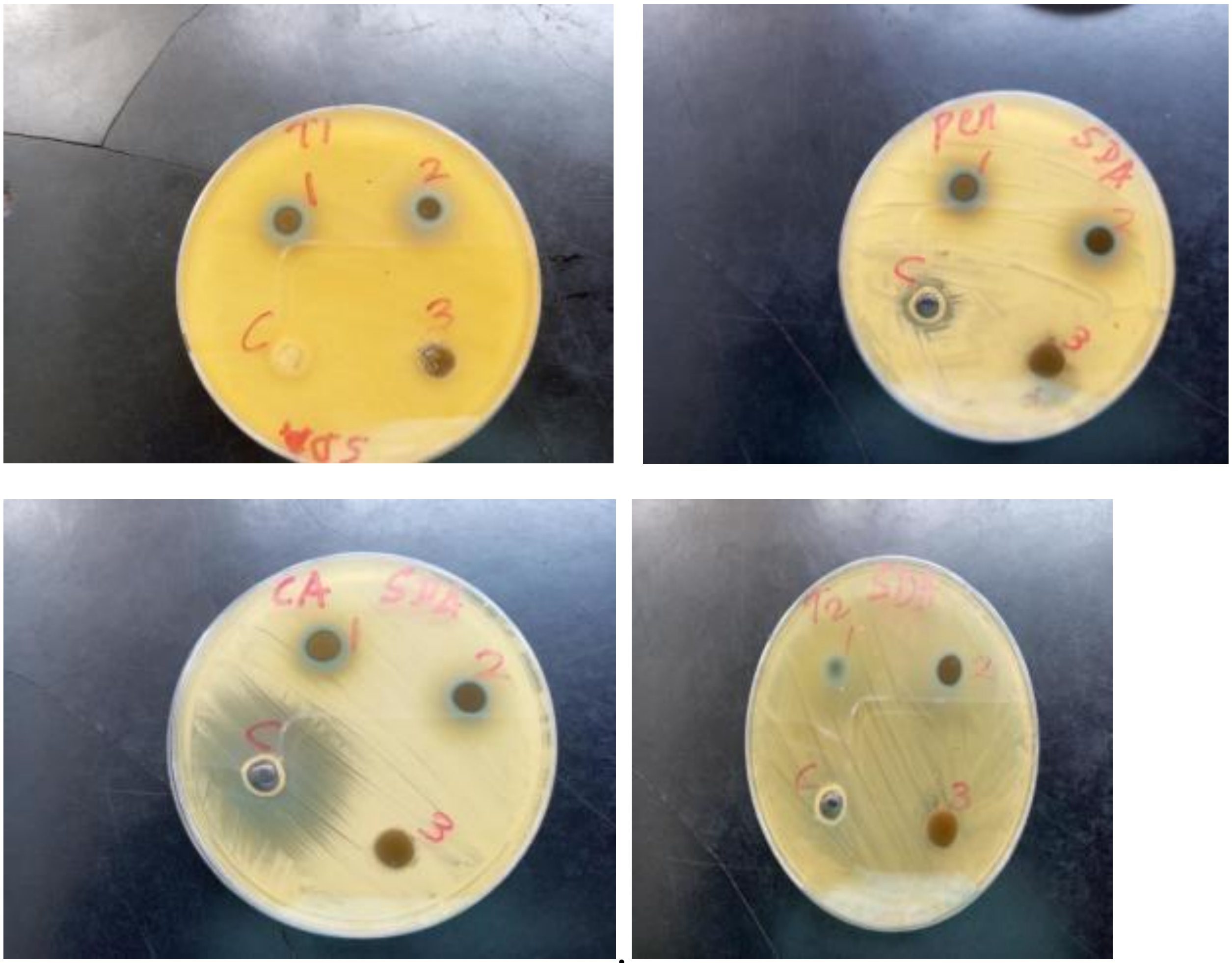
Organism	**Inference**
Trichophyton rubrum	+
Trichophyton terrestre	+
Aspergillus nidulans	+
Saprochaeta capitata	+

+: Growth

APPENDIX II







Plates showing antimicrobial susceptibility