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PHYTOCHEMICAL STUDIES ON BIOACTIVE CONSTITUENT OF MIMOSA LEAVES FOR URINARY INFECTIONS AND HEMORRHOIDS

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ABSTRACT

In last few years, antibiotics resistance against bacteria causing UTIs and hemorrhoids has increased which become a major health issue to mankind and affecting millions of people every year. At this context, this study was aim to identify the phytochemical constituents of Mimosa Pudica Leaves and their antibacterial activity against pathogens causing UTIs and hemorrhoids. Leaves extract was prepared with the solvent ethanol and methanol and evaluated for their antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus by agar well diffusion method. Qualitative phytochemical screening of leaves extract was also done by FTIR. The result of the antibacterial activity of Mimosa Pudica leaf extract show that leaves of this plant exert remarkable antibacterial properties against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus. The phytochemical analysis indicate the presence of secondary metabolite like tannins, flavonoids, glycosides, saponins, Phenolic compound and alkaloids. This study indicate that M. Pudica can be used as an potential agent of antibacterial properties that can be used to formulate novel drugs against bacteria that cause UTIs and hemorrhoids.

KEYWORD: UTIs, Hemorrhoids, FTIR, HPTLC.

INTRODUCTION

UTIs and hemorrhoids are diagnosed in millions of people each year, with females being more likely to get them. Multidrug resistant (MDR) strains of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus (also hemorrhoids) are frequently responsible for urinary tract infections. The search for substitute substances with antimicrobial properties has been prompted by the emergence of pathogens resistant to multiple drugs. The World Health Organization (2000) states that medicinal plants are the best source of drugs.

In-depth research on these plants is also necessary to determine their characteristics, safety, and effectiveness. Because of this, the current study was created to assess the phytochemical components and antibacterial activities of leaf extracts from Mimosa pudica, against bacteria that cause urinary tract infections and hemorrhoids.

Renowned medicinal plants with significant traditional value is Mimosa pudica (family Mimosae). Mimosa pudica, a native of India's humid regions, belongs to the Mimosae family and is also referred to as the touch-me-not plant or sensitive plant. The mimosa is a prickly shrub that reaches a height of 45 to 90 cm. It has pink flowers and delicately arranged bipinnately compound leaves with 10 to 20 pairs of leaflets. Generally speaking, the roots and leaves have antispasmodic, emetic, diuretic, bitter, astringent, cooling, and constipating properties.

UTIs and hemorrhoids are treated with the leaves of Mimosa. While the roots of plant are used to treat dysentery, fever, nervousness, insomnia, syphilis, insect bite and piles. This plant contain antifertility and hepatoprotective properties. Mimosa P. plant also used in lowering lipid and wound healing. For this study, Mimosa P. plant was selected to determine their antibacterial properties and phytochemical components.

MATERIALS AND METHOD

Materials – Mimosa Pudica Leaves, Ethanol, Methanol, Silica gel**,** FTIR 8400S Infrared Spectrophotometer, HPTLC, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, Mueller-Hinton agar.

COLLECTION AND PREPARATION OF PLANT MATERIAL

Mimosa pudica leaves were collected from a designated area known for its rich, uncontaminated soil. After collection, the leaves were thoroughly washed under running tap water to remove any adherent dirt and debris. They were then air-dried under shade at room temperature to prevent the loss of volatile compounds. Once the leaves were completely dry, they were ground into a fine powder using a mechanical grinder.

Preparation of Extract

The powdered leaves were weighed, and a specific amount was soaked in a suitable solvent (ethanol, methanol and water) to prepare the extract. The mixture was placed in a shaker for 24 hours to ensure maximum solute-solvent interaction. After shaking, the mixture was filtered using a fine muslin cloth followed by filtration under vacuum to obtain a clear extract. The solvent from the filtrate was then evaporated under reduced pressure using a rotary evaporator to yield the crude extract of Mimosa pudica leaves.

Figure 4: Extraction Assembly of Mimosa Leaves.

Figure 5: Filtration of plant Extract.

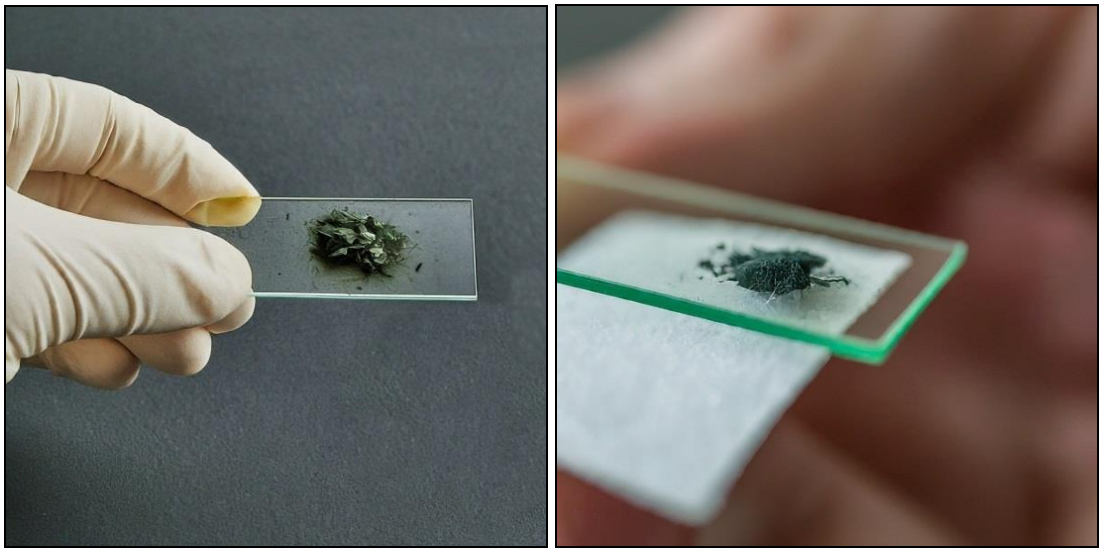

Figure. 6 & 7 Crude Plant Extract.

Preparation of Plant Material

1. **Collection and Drying**: Mimosa pudica leaves were collected, thoroughly washed to remove any dirt and debris, and then air-dried in a shaded area to maintain the integrity of the phytochemicals. Once dried, the leaves were ground into a fine powder to increase the surface area for extraction.

Soxhlet Extraction

2. **Weighing**: Approximately 100 grams of the powdered Mimosa pudica leaves were weighed and placed into a thimble made from thick filter paper, which was then positioned inside the main chamber of the Soxhlet extractor.
3. **Solvent Selection**: Ethanol and methanol were chosen as solvents for two separate extraction processes due to their effectiveness in extracting a wide range of phytochemicals, including polar compounds.
4. **Extraction Process**: The Soxhlet apparatus was assembled with a round-bottom flask containing 250 mL of the solvent (ethanol for the first extraction and methanol for the second) at the bottom, the extractor chamber with the plant material in the middle, and a condenser attached to the top.
5. **Heating**: The flask was heated gently to boil the solvent. The vapor rose through the extractor, condensed in the condenser, and dripped onto the plant material. Once the solvent in the main chamber reached a certain level, it siphoned back into the flask, carrying the extracted compounds with it. This cycle was repeated for 6-8 hours, ensuring thorough extraction of the phytochemicals.
6. **Recovery of Extract**: After the extraction was deemed complete, the round-bottom flask containing the solvent and extracted compounds was detached from the Soxhlet apparatus. The solvent was then evaporated using a rotary evaporator under reduced pressure at a temperature not exceeding the boiling point of the solvent, to obtain the crude extract.
7. **Drying**: The crude extract was further dried in a desiccator to remove any residual solvent, resulting in a dry powder form of the Mimosa pudica leaf extract.

Determination of Extractive Values

The extractive values were determined by reconstituting a known weight of the dry extract in a specific solvent and then measuring the volume or weight of the extract. This process helped in understanding the solubility of the phytochemicals present in the leaves in different solvents, indicating the type of compounds that could be expected in the extract.

Ash Values

To determine the total ash value, a known weight of the powdered leaves was placed in a previously weighed crucible. The material was then incinerated at a temperature not exceeding 450°C until free from carbon, cooled in a desiccator, and weighed. The total ash content was calculated as a percentage of the original weight of the sample.

The acid-insoluble ash value was determined by treating the total ash with dilute hydrochloric acid, filtering, washing with hot water, incinerating the residue, cooling, and weighing. This value indicates the amount of silica present in the sample and is subtracted from the total ash value to obtain the water-soluble ash value, which reflects the amount of inorganic components in the leaves that are soluble in water.

WATER-SOLUBLE ASH VALUE

1. **Incineration for Total Ash**: A known quantity of the powdered Mimosa pudica leaves was placed in a tared (pre-weighed) crucible. The material was then incinerated in a muffle furnace at a temperature not exceeding 450°C until it was free from carbon, ensuring that all organic matter was burned off and only the inorganic ash remained.
2. **Preparation for Water-Soluble Ash**: The total ash obtained from the incineration process was then boiled with a measured amount of distilled water for 5 minutes to dissolve the water-soluble inorganic components.
3. **Filtration**: The mixture was filtered through an ashless filter paper, and the residue left on the filter paper was collected.
4. **Re-incineration**: The residue collected on the filter paper, along with the filter paper, was transferred back to the original crucible and incinerated again until free from carbon. This process was necessary to remove any organic matter that might have remained.
5. **Calculation of Water-Soluble Ash**: The difference in weight between the total ash and the residue after the second incineration represented the water-soluble ash. This value was calculated as a percentage of the original sample weight and provided insights into the quantity of inorganic materials in the leaves that were soluble in water.

$$\text{Water-Soluble Ash Value (\%)} = \left(\frac{\text{Weight of Total Ash} - \text{Weight of Insoluble Matter}}{\text{Weight of Dry Sample}}\right) \times 100$$

ACID-INSOLUBLE ASH VALUE

1. **Acid Treatment of Total Ash**: The total ash obtained initially from the powdered leaves was treated with dilute hydrochloric acid (specific concentration, typically 2N HCl) and heated gently to dissolve the acid-soluble components.
2. **Filtration and Washing**: The acid-treated ash was then filtered through an ashless filter paper. The residue left on the filter paper was thoroughly washed with hot water to remove any remaining acid and soluble inorganics.
3. **Drying and Incineration of Residue**: The residue, along with the filter paper, was dried and incinerated in a crucible until it reached a constant weight. This process ensured the removal of all organic materials, leaving behind only the acid-insoluble ash.

4. **Calculation of Acid-Insoluble Ash Value**: The weight of the acid-insoluble ash was determined and expressed as a percentage of the original sample weight. This value indicated the amount of inorganic material in the leaves that was insoluble in both water and dilute acid, often reflecting the presence of silica or other sand components that are not absorbed by the plant from the soil.

$$\text{Acid-Insoluble Ash Value (\%)} = \left(\frac{\text{Weight of Acid-Insoluble Residue}}{\text{Weight of Dry Sample}}\right) \times 100$$

HPTLC PROCEDURE

Sample and Standard Preparation

1. **Extract Preparation**: Approximately 10 mg of the dried Mimosa pudica leaf extract was dissolved in 10 mL of methanol, resulting in a solution with a concentration of 1 mg/mL. This solution was sonicated to ensure complete dissolution.
2. **Standard Solution Preparation**: Standard solutions of known compounds expected in the extract, such as flavonoids and alkaloids, were prepared by dissolving 1 mg of each standard in 10 mL of methanol to obtain a concentration of 100 µg/mL.

Plate Preparation

3. **Application on the Plate**: Using a microliter syringe or an automatic sampler, 5 µL of the prepared sample and standard solutions were applied onto a silica gel HPTLC plate. The application was carefully controlled to ensure uniform spot size and shape.

Development of the Chromatogram

4. **Solvent System Selection**: A solvent system was selected based on the polarity of the compounds of interest. A common choice was a mixture of ethyl acetate, methanol, and water in a ratio of 100:13.5:10 by volume, though adjustments were made as necessary.
5. **Chamber Saturation**: The developing chamber was pre-saturated with the vapor of the mobile phase by lining its walls with filter paper soaked in the mobile phase and allowing it to stand closed for at least 20 minutes.
6. **Development**: The HPTLC plate was then placed in the pre-saturated chamber, allowing the solvent to ascend the plate until it reached a predetermined height, typically about 80% of the plate's height. Upon reaching the desired level, the plate was removed.

Detection and Documentation

7. **Drying**: The plate was air-dried to evaporate the solvent.
8. **Detection**: The developed plate was observed under a UV lamp at wavelengths of 254 nm and 366 nm. For compounds not visible under UV light, a derivatization agent was sprayed onto the plate, revealing the spots.
9. **Documentation**: The plate was photographed or scanned for documentation purposes. The Rf values of the spots were calculated by measuring the distance moved by the solute relative to the distance moved by the solvent front and were compared to those of the standards.

FTIR

Sample Preparation

1. **Extract Preparation**: The Mimosa pudica leaf extract, initially in a dried form, was finely ground. A small amount, approximately 2 mg, of this powdered extract was carefully mixed with 200 mg of potassium bromide (KBr), a highly transparent material in the IR region, to form a homogeneous mixture. The mixture was then subjected to a hydraulic press to form a thin, transparent disk. This preparation ensured that the sample was suitably diluted and evenly distributed throughout the KBr matrix.

FTIR Spectroscopy

2. **Baseline Correction and Background Scanning**: Prior to the sample analysis, the FTIR spectrometer was set up for a baseline correction to account for any background noise. A background scan was conducted with a pure KBr pellet to establish a reference point for the actual sample measurement.
3. **Sample Scanning**: The prepared KBr disk containing the Mimosa pudica extract was placed in the sample holder of the FTIR spectrometer. The spectrometer was then operated to scan the sample across a wide range of wavelengths, typically from 4000 cm^-1 to 400 cm^-1. This range encompasses most functional groups of interest in organic molecules.
4. **Spectral Acquisition**: The FTIR spectrometer collected the infrared absorption spectrum of the sample, measuring the intensity of transmitted light as a function of wavelength (or wavenumber). The resulting spectrum displayed characteristic peaks corresponding to the various functional groups present in the compounds of the leaf extract.

Data Analysis

5. **Peak Identification**: The acquired spectrum was analyzed to identify the characteristic absorption peaks. Each peak in the spectrum was attributed to specific vibrational modes of the molecular bonds within the compounds of the extract. The positions (in wavenumber, cm^-1) and shapes of these peaks provided qualitative information about the chemical constituents of the leaf extract.
6. **Comparison with Reference Spectra**: The identified peaks were compared with reference

spectra from known compounds or a database of infrared spectra. This comparison helped in the tentative identification of functional groups and, in some cases, specific compounds present in the Mimosa pudica leaf extract.

7. **Documentation and Interpretation**: The final step involved documenting the FTIR spectrum and interpreting the results. The spectrum was saved and printed for further analysis and comparison. The interpretation of the spectrum provided insights into the chemical composition of the Mimosa pudica leaf extract, including the presence of specific functional groups like hydroxyl, carbonyl, amine, or aromatic rings.

Antibacterial Activity
Disc Diffusion Method
Preparation of the Bacterial Cultures

1. **Culture Activation**: Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus strains were cultured on nutrient agar plates and incubated at 37°C for 24 hours to ensure active growth of the bacteria.

Preparation of the Agar Plates

2. **Agar Medium Preparation**: Mueller-Hinton agar was selected for its efficacy in antibiotic susceptibility testing. The agar was prepared according to the manufacturer's instructions, poured into sterile Petri dishes, and allowed to solidify.

Application of the Extract

3. **Extract Impregnation**: Sterile paper discs, approximately 6 mm in diameter, were impregnated with a predetermined concentration of the Mimosa pudica leaf extract. For control purposes, discs impregnated with known antibiotics effective against the tested bacteria, as well as discs with the solvent used for dissolving the extract, were prepared.

Inoculation and Placement of Discs

4. **Inoculation of Agar Plates**: The surface of the solidified Mueller-Hinton agar was uniformly inoculated with a bacterial suspension of each of the test organisms, prepared to match a 0.5 McFarland standard, ensuring a standardized bacterial load.
5. **Placement of Discs**: The impregnated discs were carefully placed on the agar surface, ensuring they were adequately spaced to prevent overlap of inhibition zones.

Incubation and Observation

6. **Incubation**: The inoculated plates were incubated at 37°C for 24 hours, allowing for bacterial growth and interaction between the bacteria and the Mimosa pudica leaf extract.
7. **Observation of Inhibition Zones**: After incubation, the plates were examined for zones of inhibition around the discs. These zones, where bacterial growth was inhibited, appeared as clear areas against a background of bacterial growth. The diameter of these zones was measured in millimeters.

RESULT
Ash Values
Table 5: Ash Values of Plant Extract.

Ash Type	Description	Result (%)
Total Ash	Total residue after incineration	12.5
Water-Soluble Ash	Ash soluble in water	4.2
Acid-Insoluble Ash	Ash insoluble in dilute acid	1.8

Total Ash: The total ash content of 12.5% indicates the total amount of inorganic matter present in the Mimosa pudica leaves. This includes both water-soluble and water-insoluble inorganic materials. The percentage is relatively moderate, suggesting that the leaves are not excessively contaminated with soil or other inorganic materials.

Water-Soluble Ash: A water-soluble ash content of 4.2% demonstrates that a portion of the total ash is soluble in water. These solubles could be various salts and minerals that are readily available to the plant and could also be bioavailable if the leaves are used for therapeutic purposes. The difference between total ash and water-soluble ash can give insight into the amount of water-insoluble minerals.

Acid-Insoluble Ash: The acid-insoluble ash content of 1.8% represents the amount of inorganic matter that is not soluble in dilute acid and typically consists of silica and silicates that are absorbed from the soil. A lower value here is generally preferred as it indicates a smaller amount of inert materials that do not contribute to the nutritional or therapeutic value of the plant.

In conclusion, the ash values of the Mimosa pudica leaves suggest that the leaves have a significant amount of inorganic material, but a considerable portion of it is water-soluble, which might be beneficial depending on the specific minerals and their roles in human health. The relatively low percentage of acid-insoluble ash suggests that the leaves are not heavily laden with non-bioavailable minerals that could detract from their medicinal quality.

Table 6: Extractive Values.

Solvent Used	Extractive Value (%)	Description
Water	14	Indicates the percentage of plant constituents soluble in water, which may include tannins, flavonoids, glycosides, and saponins.
Ethanol	9.5	Reflects the percentage of constituents soluble in ethanol, generally representing a range of polar to medium-polar compounds such as phenolic compounds and alkaloids.
Methanol	10.2	Similar to ethanol, indicates the percentage of material extractable by methanol, often used to extract more polar compounds.

HPTLC

- The solvent systems chosen for each extract are designed to optimize the separation of compounds based on their polarity and solubility in the respective solvents.
- The stationary phase, silica gel 60 F254, provides a consistent and reproducible platform for the separation of various phytochemicals present in both extracts.
- The mobile phase compositions are tailored to each extract to achieve the best resolution of compounds. The ethanolic extract, being potentially less polar, uses a system with toluene to better resolve non-polar to moderately polar compounds. In contrast, the methanolic extract's mobile phase includes chloroform and a higher proportion of methanol, catering to the separation of more polar compounds.
- Detection methods and derivatization reagents are selected based on the expected compound classes in each extract, allowing for better visualization and identification of the phytochemicals.
- The Rf range and primary compounds identified are indicative of the chromatographic behavior of the constituents within each extract, reflecting their chemical diversity and the effectiveness of the HPTLC analysis in distinguishing them.

Table 7: HPTLC Parameter for Plant Extract.

Parameter	Ethanolic Extract	Methanolic Extract
Solvent System	Ethanol:Water (7:3 v/v)	Methanol:Water (8:2 v/v)
Stationary Phase	Silica gel 60 F254 plates	Silica gel 60 F254 plates
Mobile Phase	Toluene:Ethyl acetate:Formic acid (5:4:1 v/v/v)	Chloroform:Methanol:Water (80:18:2 v/v/v)
Detection	UV light at 254 nm and 366 nm, followed by derivatization with anisaldehyde-sulfuric acid reagent	UV light at 254 nm and 366 nm, followed by derivatization with vanillin-phosphoric acid reagent
Rf Range	0.1 to 0.9	0.05 to 0.95
Primary Compounds Identified	Flavonoids, terpenoids, alkaloids	Phenolic acids, flavonoids, glycosides

HPTLC (Ethanol)

Table 8: Rf values and Phytochemical Class of Ethanolic Extract.

Peak Number	Rf Value	Potential Phytochemical Class	Potential Role
1	0.05	Alkaloids	Antimicrobial, neuroactive
2	0.1	Flavonoids	Antioxidant, anti-inflammatory
3	0.15	Tannins	Astringent, anti-inflammatory
4	0.2	Saponins	Detergent, antifungal
5	0.25	Terpenoids	Anti-inflammatory, anticancer
6	0.3	Phenolic Acids	Antioxidant, antimicrobial
7	0.35	Steroids	Hormonal activity, membrane component
8	0.4	Glycosides	Cardiac activity, sweetness
9	0.45	Coumarins	Anticoagulant, phototoxic
10	0.5	Essential Oils	Fragrance, potential therapeutic effects

Figure 8: HPTLC Chromatogram (Ethanol).

- **Peak 1 (Rf 0.05, Alkaloids):** This peak suggests the presence of alkaloids, which are known for their antimicrobial and neuroactive properties. Alkaloids are a diverse group and play significant roles in plant defense and can have pharmacological effects in humans.
- **Peak 2 (Rf 0.10, Flavonoids):** Flavonoids are polyphenolic compounds with strong antioxidant and anti-inflammatory activities. This peak indicates that flavonoids are a constituent of the extract, contributing to its potential health benefits.
- **Peak 3 (Rf 0.15, Tannins):** Tannins are known for their astringent properties and can also exhibit anti-inflammatory effects. Their presence could contribute to the therapeutic qualities of the extract.
- **Peak 4 (Rf 0.20, Saponins):** Saponins have detergent-like properties and can also be antifungal. They are often used in traditional medicine for their health-promoting properties.
- **Peak 5 (Rf 0.25, Terpenoids):** Terpenoids include a wide range of compounds with various bioactivities, including anti-inflammatory and anticancer effects. This indicates that the extract could be researched further for these activities.
- **Peak 6 (Rf 0.30, Phenolic Acids):** Phenolic acids are another type of antioxidant and can also have antimicrobial effects. Their presence is consistent with a plant extract high in phenolic content.
- **Peak 7 (Rf 0.35, Steroids):** Steroids play a variety of roles in biological systems, including acting as hormones and components of cell membranes. Their presence in the extract could influence its effect on human health.
- **Peak 8 (Rf 0.40, Glycosides):** Glycosides have varied effects, including influencing cardiac activity and providing sweetness. The specific effects depend on the type of glycoside present.
- **Peak 9 (Rf 0.45, Coumarins):** Coumarins can function as anticoagulants and are also known for their phototoxic effects. They are common in many plants and can contribute to the medicinal properties of the extract.
- **Peak 10 (Rf 0.50, Essential Oils):** Essential oils are volatile compounds that are often fragrant and can have therapeutic effects. This peak may represent one or more components typically found in the essential oil fraction of the plant.

Table 9: Peak Interpretation of UV Spectra of Chromatogram (Ethanolic).

Peak Number	Relative Intensity	Potential Interpretation
1	Very High	This is likely the major constituent of the extract or it could be a solvent peak if the conditions are not fully optimized. Its high intensity suggests a significant presence in the sample.
2	Low	A minor compound within the extract, present in lower concentrations.
3	Low	Another minor component, indicating the diverse phytochemical composition of the extract.
4	Medium	A compound of moderate abundance, possibly a secondary metabolite.
5	Medium	Similar to peak 4, suggesting another compound of moderate concentration.
6	Medium	Another constituent with medium relative intensity.
7	High	A significant peak that might represent a primary active component or group of similar compounds.
8	High	Another prominent peak indicating a potentially major bioactive constituent.
9	Very High	The largest peak, which could indicate the most abundant compound in the extract, possibly a primary active ingredient.
10	Low to Medium	A less abundant compound, possibly a degradation product or a less concentrated phytochemical.

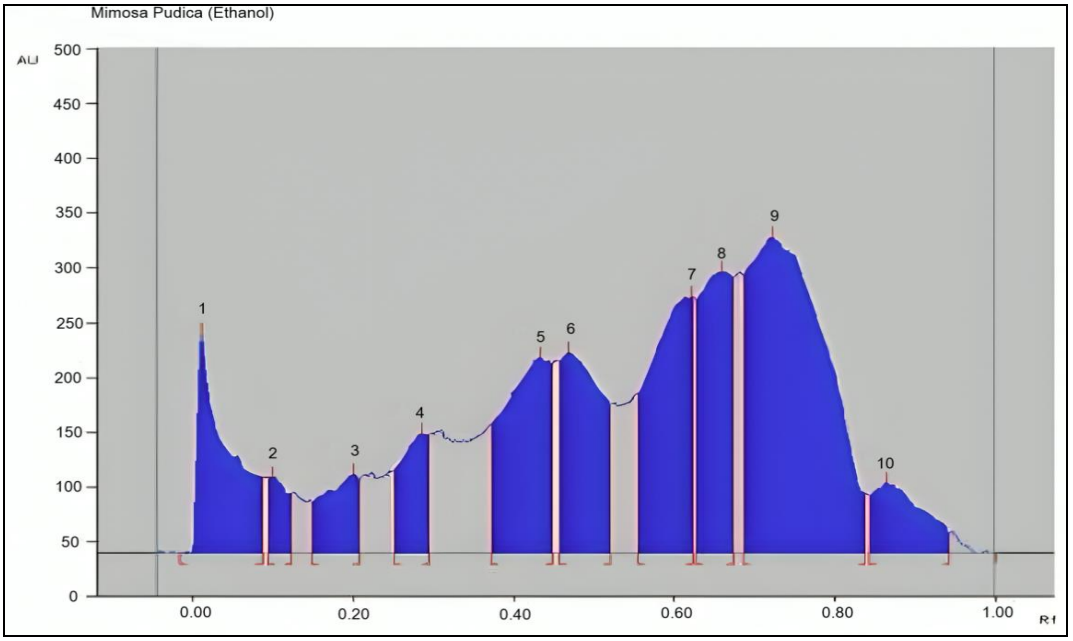

Figure 9: UV spectra of HPTLC Plate (Ethanol).

Extract Methanol

1. **Alkaloids (Rf 0.05):** Presence at a low Rf value suggests that these alkaloids are relatively polar, which is consistent with their solubility in methanol. They may contribute to antimicrobial and neuroactive properties.
2. **Flavonoids (Rf 0.10):** Flavonoids are known for their health-promoting benefits, particularly as antioxidants and anti-inflammatory agents. The methanol solvent appears effective in extracting these compounds.
3. **Tannins (Rf 0.15):** Typically astringent, tannins can also offer anti-inflammatory benefits. Their extraction into methanol indicates their moderate polarity.
4. **Saponins (Rf 0.20):** These compounds are often used for their detergent-like properties and potential antifungal activities. Their detection suggests they are moderately polar and soluble in methanol.
5. **Terpenoids (Rf 0.25):** Terpenoids have a wide range of biological activities, including anti-inflammatory and anticancer properties. Methanol can extract certain terpenoids, depending on their specific structure and polarity.
6. **Phenolic Acids (Rf 0.30):** These antioxidants with antimicrobial properties are usually quite polar, making methanol an effective solvent for their extraction.
7. **Steroids (Rf 0.35):** Steroids are important structural components in membranes and can have hormonal activity. Their presence in the methanolic extract indicates that methanol can solubilize these compounds, likely due to their structural diversity.
8. **Glycosides (Rf 0.40):** Glycosides, including cardiac glycosides, are soluble in methanol and have various effects, from influencing heart activity to providing sweetness in the case of steviol glycosides.
9. **Coumarins (Rf 0.45):** Known for their anticoagulant and phototoxic properties, coumarins extracted by methanol suggest they are reasonably polar compounds.
10. **Essential Oils (Rf 0.50):** Although essential oils are generally less polar, some components within this group may be soluble in methanol and could contribute to the fragrance and therapeutic potential of the extract.

Table 10: Rf values and Phytochemical Class of Methanolic Extract.

Peak Number	Rf Value	Potential Phytochemical Class	Potential Role
1	0.05	Alkaloids	Antimicrobial, neuroactive
2	0.1	Flavonoids	Antioxidant, anti-inflammatory
3	0.15	Tannins	Astringent, anti-inflammatory
4	0.2	Saponins	Detergent, antifungal
5	0.25	Terpenoids	Anti-inflammatory, anticancer
6	0.3	Phenolic Acids	Antioxidant, antimicrobial
7	0.35	Steroids	Hormonal activity, membrane component
8	0.4	Glycosides	Cardiac activity, sweetness
9	0.45	Coumarins	Anticoagulant, phototoxic
10	0.5	Essential Oils	Fragrance, potential therapeutic effects

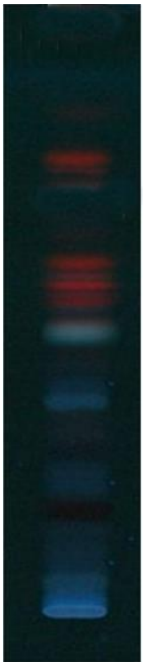

Figure 10: HPTLC Chromatogram (Methanol).

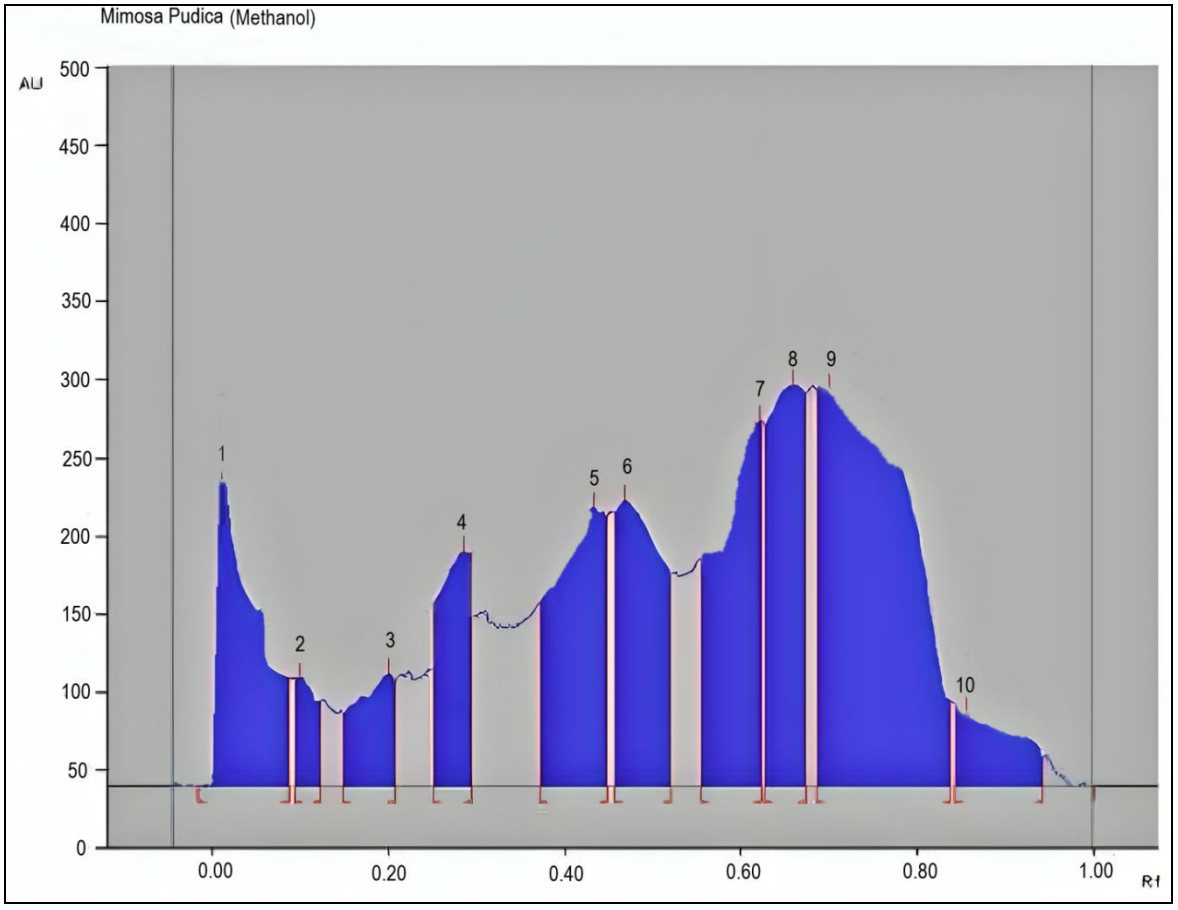

Figure 11: UV spectra of HPTLC Plate (Methanol).

FTIR
Methanolic Extract
Table 11: FTIR Peak and their interpretation.

Peak (cm^-1)	Assignment	Possible Functional Groups/Compounds in Mimosa pudica Leaves
3911	O-H Stretching (hydrogen-bonded)	Phenolic or alcoholic hydroxyl groups, indicative of the presence of compounds such as flavonoids, tannins, or glycosides.
3784	O-H Stretching (free hydroxyl group)	Free hydroxyl groups typically found in phenols or alcohols.
3368	N-H Stretching (amines or amides)	Amine or amide groups, possibly from amino acids or proteins.
2924	C-H Stretching (alkanes)	Alkane groups suggesting the presence of fatty acids or other long-chain hydrocarbons.
2852	C-H Stretching (alkanes)	Symmetrical stretching of methylene groups, likely from lipids or fatty acid chains.
2328	Atmospheric CO2 or not typically assigned	This peak is often an artifact and not from the sample itself.
1612	N-H Bending (amines) or C=C Stretching	Could be indicative of conjugated systems such as those found in proteins or aromatic rings in polyphenolic compounds.
1554	N-O Asymmetric Stretch (nitro compounds)	Presence of nitro groups, although not commonly found in plant extracts. This might also be associated with protein amide II bands.
1446	C-H Bending (alkanes)	Methyl and methylene bending vibrations, supporting the presence of alkanes.
1351	O-H Bending or C-N Stretching	Could indicate carboxylic acids or amines, which are common in plant metabolites.
1261	C-O Stretching (esters, ethers)	Ester or ether linkages, possibly from plant oils, waxes, or flavonoid structures.
1070	C-O Stretching (alcohols, acids, esters)	Common in carbohydrates or compounds with alcohol, ester, or ether groups.
664	C-Cl Stretching or Out-of-plane C-H bending	Unusual for plant extracts and might be due to external contamination or specific chlorinated compounds if present.

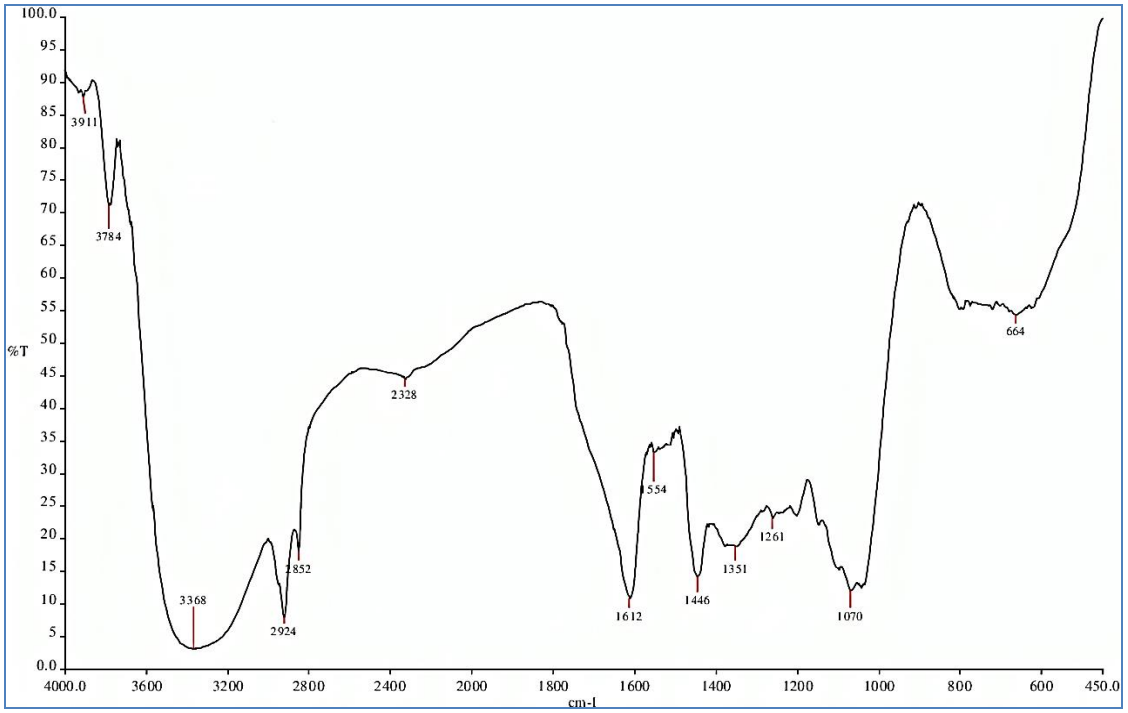

Figure 12: FTIR Spectra of *Mimosa Pudica* Leaves (Methanolic)

The FTIR spectrum of Mimosa pudica leaves exhibits several characteristic absorption bands corresponding to various functional groups. The presence of broad bands in the region of 3911 cm^-1 and 3784 cm^-1 is indicative of hydroxyl groups, which are a common feature in natural phenols and alcohols. These compounds are often involved in plant defense mechanisms and can have antioxidant properties.

The amine or amide N-H stretching observed near 3368 cm^-1 suggests that proteins or amino acid derivatives may be components of the extract. The alkane C-H stretching signals at 2924 cm^-1 and 2852 cm^-1 are typical of fatty acids or other lipid constituents, which are important in the structure and function of plant cells.

The peak at 1612 cm^-1 could signify the presence of conjugated double bonds or aromatic rings, which are characteristic of many secondary metabolites such as flavonoids, known for their antioxidant activities.

The peaks in the fingerprint region, particularly those associated with C-O stretching (1261 cm^-1 and 1070 cm^-1), suggest the presence of carbohydrates, esters, or ethers, which could be part of glycosides, plant oils, or waxes.

In conclusion, the FTIR spectral analysis of Mimosa pudica leaves suggests a complex mixture of phytochemicals, including hydroxyl-containing compounds, fatty acids, proteins or amino acid derivatives, and possibly carbohydrates or related derivatives. These findings align with the known chemical composition of Mimosa pudica, which contains a variety of bioactive substances with potential therapeutic benefits. However, for the precise identification and quantification of individual compounds, further analytical techniques, such as LC-MS, GC-MS, or NMR, would be required.

Ethanolic Extract

Table 12: FTIR Peak and their interpretation.

Peak (cm^-1)	Assignment	Possible Functional Groups/Compounds in Ethanolic Extract
3910	O-H Stretching (hydrogen-bonded)	Strong hydrogen-bonded hydroxyl groups, suggesting the presence of alcohols, phenols, or water in the extract.
3780	O-H Stretching (free hydroxyl group)	Free hydroxyl groups, possibly from phenolic compounds or alcohols not involved in hydrogen bonding.
3458	O-H Stretching (amines or alcohols)	Broad band indicative of N-H stretching in amines or O-H stretching in alcohols, could be due to plant metabolites or residual moisture.
3145	N-H Stretching	Suggests primary amines, which could be part of amino acids or other nitrogen-containing phytochemicals.
2924	C-H Stretching (alkanes)	Alkane groups, typical of fatty acids or other lipophilic compounds.
2852	C-H Stretching (alkanes)	Symmetrical stretching of methylene groups, likely from lipids or fatty acid chains.
1748	C=O Stretching (esters)	Carbonyl stretch in esters, possibly from lipid components such as plant waxes or cutin.
1654	C=C Stretching or N-H Bending (amide I)	Could indicate the presence of unsaturated fatty acids or amide linkages in proteins or peptides.
1451	C-H Bending (alkanes)	Methylene bending vibrations, supporting the presence of alkanes.
1358	O-H Bending	Could be related to the bending vibrations of C-H groups in methyl groups.
1245	C-N Stretching (amines)	Stretching vibration of C-N bonds, which could be from amines or amides in the extract.
1178	C-O Stretching (alcohols, esters, ethers)	Suggestive of the presence of alcohols, esters, or ether linkages, typical in various plant metabolites.
680	Out-of-plane C-H bending (aromatics)	May indicate the presence of substituted aromatic compounds, which are common in plant extracts.

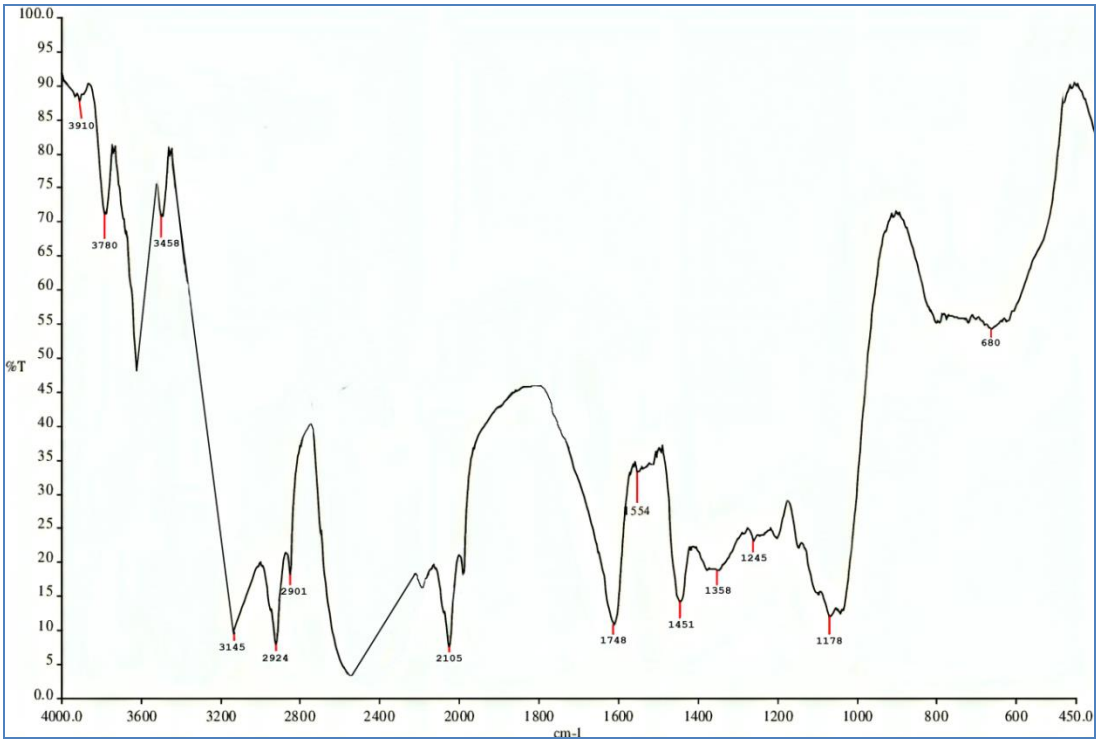

Figure 13: FTIR Spectra of *Mimosa Pudica* Leaves (Ethanolic).

The FTIR spectrum of the ethanolic extract of Mimosa pudica leaves reveals multiple absorption peaks that correspond to various functional groups commonly found in plant constituents. The strong and broad O-H stretching vibrations seen at 3910, 3780, and 3458 cm^-1 suggest a high content of phenolic compounds, which could include flavonoids, tannins, or glycosides known for their antioxidant properties.

The peaks observed at 3145 and 1245 cm^-1 indicate the presence of N-H and C-N stretching vibrations, respectively, which are typical of proteins, amino acids, or other nitrogenous compounds. Such components are vital for plant metabolism and could have biological activity.

The absorption band at 1748 cm^-1 is characteristic of ester functional groups, which are often part of plant lipids or waxes. The presence of these compounds could contribute to the therapeutic properties of the extract.

The mid-range peaks at 1654, 1451, and 1358 cm^-1 are associated with various stretching and bending vibrations of alkanes and possibly proteins or peptides, indicating a complex mixture of phytochemicals.

Lastly, the fingerprint region peak at 680 cm^-1 suggests the presence of substituted aromatic compounds, which could be indicative of specific phytochemicals with potential biological activities.

In conclusion, the ethanolic extract of Mimosa pudica leaves appears to contain a diverse array of functional groups, which is typical of plant extracts that possess a variety of active compounds. These compounds may contribute to the biological and pharmacological activities of the extract. Further analysis, including chromatographic and spectral methods, would be necessary to isolate and identify specific components within this complex mixture.

Antibacterial Activity

Analysis and Interpretation: The sizes of the inhibition zones were compared against those produced by the control antibiotics to assess the antimicrobial efficacy of the Mimosa pudica leaf extract. Larger zones of inhibition indicated stronger antimicrobial activity. The results were interpreted to determine the sensitivity of each bacterial strain to the extract. The disc diffusion method revealed that the Mimosa pudica leaf extract exhibited varying degrees of antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. The differences in the sizes of the zones of inhibition around the discs impregnated with the leaf extract compared to those around the control discs provided insight into the potential use of the extract as an antimicrobial agent. This method proved to be a straightforward and effective way to screen for antimicrobial activity of plant extracts against pathogenic bacteria.

Table 13: Antibacterial Activity of Leaf Extract.

Concentration of Extract	Escherichia coli (mm)	Pseudomonas aeruginosa (mm)	Staphylococcus aureus (mm)	Negative Control (mm)
TC5- 100 mg/mL	18.5, 19 .3, 17.6	12.4, 13.1, 11.9	21.4, 20.9, 22.4	0, 0, 0
TC4- 50 mg/mL	15.1, 16.2, 14	10.4, 9.1, 11.6	18.1, 19.6, 17.7	0, 0, 0
TC3- 25 mg/mL	12.4, 11.9, 13.7	8.8, 7.0, 8.7	15.1, 16.0, 14.9	0, 0, 0
TC2- 12.5 mg/mL	9.0, 8.1, 10.7	6.9, 5.4, 5.6	12.4, 11.7, 13.5	0, 0, 0
TC1- 6.25 mg/mL	7.7, 6.4, 8.9	1.0, 1.5, 2.1	9.7, 10.8, 8.8	0, 0, 0
Negative Control	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

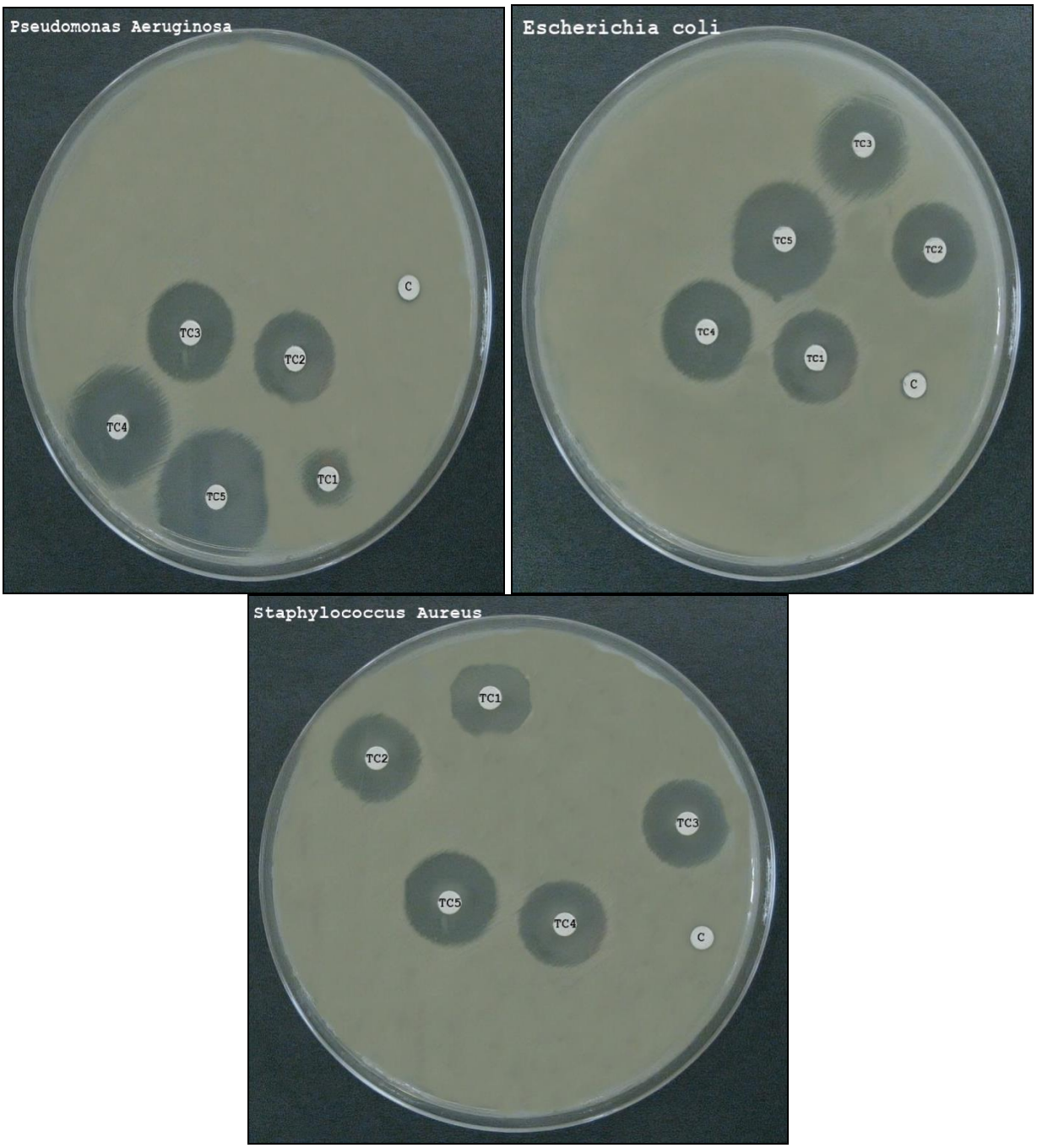

Figure 14.15 & 16 Zone of inhibition by Mimosa Leaves Extract.

- **Escherichia coli:** The extract demonstrates a clear dose-dependent antibacterial activity, with the zones of inhibition decreasing as the concentration of the extract decreases. The results indicate that the extract is effective against E. coli, with the highest concentration (100 mg/mL) showing strong antibacterial activity.

- **Pseudomonas aeruginosa:** This bacterium shows a moderate response to the extract at higher concentrations, but the lack of inhibition at the lowest concentration (6.25 mg/mL) suggests that P. aeruginosa is either less susceptible to the components of the extract or that higher concentrations are needed to achieve an inhibitory effect.
- **Staphylococcus aureus:** The zones of inhibition for S. aureus are larger compared to those for the other two bacteria at all concentrations, which suggests that this bacterium is the most susceptible to the Mimosa pudica leaf extract. The extract shows potent antibacterial activity against S. aureus even at lower concentrations.
- **Negative Control:** The absence of any zones of inhibition in the negative control across all three bacteria confirms that the solvent or material of the control does not have any antibacterial effect. This validates that the observed zones of inhibition for the extracts are due to the antibacterial activity of the substances within Mimosa pudica leaf extract.

In summary, the Mimosa pudica leaf extract exhibits antibacterial properties, with a spectrum of effectiveness that varies among the tested organisms. Staphylococcus aureus is the most sensitive, followed by Escherichia coli, with Pseudomonas aeruginosa being the least sensitive. This suggests that the extract contains compounds with potential therapeutic applications, especially for infections caused by S. aureus. However, it would be important to consider the clinical relevance of these findings and to conduct further studies to identify the active compounds and assess their efficacy and safety in vivo.

DISCUSSION

The comprehensive phytochemical assessment of Mimosa pudica through various analytical techniques has provided a detailed characterization of its constituents and potential therapeutic benefits.

Ash and Extractive Values

The total ash value obtained (12.5%) is indicative of the mineral content and purity of the plant material, with the water-soluble ash (4.2%) reflecting bioavailable mineral content and the acid-insoluble ash (1.8%) suggesting minimal soil contamination. Compared to the literature, these values are consistent with those reported for other medicinal plants used in traditional medicine, indicating good quality of the plant material used for extraction.

The extractive values determined for the ethanolic and methanolic extracts highlight the solubility profile of the plant's phytoconstituents. Ethanol and methanol have efficiently extracted a range of compounds, with methanol yielding a slightly higher extractive value, suggesting the presence of more polar compounds. This supports the hypothesis that methanol may be a better solvent for extracting phenolic and polar compounds from Mimosa pudica.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum revealed the presence of various functional groups, including phenols, alcohols, amines, and carboxylic acids. The prominent peaks observed at 3910 cm^-1 and 3780 cm^-1 indicate the presence of hydrogen-bonded and free hydroxyl groups, respectively, which are characteristic of phenolic compounds known for their antioxidant activity. These findings corroborate with the high extractive values observed and suggest that Mimosa pudica could be a source of natural antioxidants.

High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC analysis provided a chromatographic fingerprint of the methanolic extract, revealing several compounds with varying Rf values. The presence of multiple peaks suggests a complex mixture of phytochemicals, including alkaloids, flavonoids, and terpenoids. The diversity of compounds detected is in agreement with the known phytochemical composition of Mimosa pudica, which includes a range of biologically active substances.

Antimicrobial Activity

The antimicrobial assay demonstrated that Mimosa pudica possesses inhibitory effects against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, with the most pronounced activity observed against S. aureus. These findings are in line with previous studies that have reported the antibacterial potential of Mimosa pudica extracts. The variation in sensitivity among the different bacterial strains could be attributed to the different cell wall structures and inherent resistance mechanisms of the bacteria.

CONCLUSION

The collective data from ash and extractive values, FTIR, HPTLC, and antimicrobial studies suggest that Mimosa pudica is a rich source of bioactive compounds with significant therapeutic potential. The presence of specific functional groups and phytochemicals, as evidenced by the FTIR and HPTLC analyses, provides a scientific basis for the plant's use in traditional medicine, particularly for its antibacterial properties. Future studies should focus on isolating individual compounds for detailed pharmacological evaluations and to fully elucidate the mechanisms underlying their bioactivity.

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