PROJECT WORK

Report on

Study of Antimicrobia Activity of Mimosa Pudica In Vitro Method

In the Faculty of Pharmacy,
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BACHELOR OF PHARMACY

Submitted by

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CERTIFICATE

This is to certify that **Maharudra Sukhadev Kharat** PRN 2125551823048 has carried out the required practice school work prescribed by Dr. Babasaheb Ambedkar Technological University, Lonere for the VIIIth semester of B. Pharm. course during academic year 2024-2025 & this report represents his/her work done under my supervision.

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ABSTRACT

This research paper aims to investigate the in vitro antimicrobial activity of Mimosa pudica, commonly known as "Sensitive Plant" or "Touch-Me-Not." Mimosa pudica is a perennial herbaceous plant traditionally used in various medicinal practices. The study utilizes different solvent extracts of Mimosa pudica to evaluate its potential as an antimicrobial agent against a range of pathogenic microorganisms. The in vitro methods employed include agar well diffusion and broth microdilution assays to determine the effectiveness of the plant extracts against various bacterial and fungal strains.

Mimosa pudica plant also called sensitive plant, is a creeping annual and perennial herb. This plant which folds itself when touched and spreads its leaves once again after a while. The plant extracts from various parts of Mimosa pudica exhibits significant antimicrobial activities against a wide range of food borne pathogens. Mimosa pudica also used to avoid or cure several disorders like cancer, diabetes, hepatitis, obesity and urinary infections. A wide array of pharmacological properties like antioxidant, antifungal, antibacterial, antidepressant and etc. have been attributed to different parts of M.pudica. The whole plant of Mimosa pudicais very useful for various pharmacological and biological activities.

1. Introduction

Mimosa pudica has been recognized for its therapeutic properties in traditional medicine, and recent scientific studies have focused on exploring its bioactive compounds. The plant is known for its diverse chemical composition, including alkaloids, flavonoids, tannins, and other secondary metabolites. This study aims to contribute to the understanding of Mimosa pudica's antimicrobial potential, which could have implications for the development of novel antimicrobial agents For a long period of time plants have been a source of medicinal agents and that natural products used to maintain the human health. Although popularity of herbal medicine are sources of bioactive phyto compounds and they are used as therapeutic agents as antimicrobial agents. Herbal medicines are gaining growing interest because of their cost effectiveness, eco-friendly attributes and true relief from disease conditions(Tomar, Shrivastava and Kaushik, 2014). Now a day multiple drug resistance has developed using the plant derived antimicrobials drugs due to the indiscriminate used and that are considered to be safer compared with synthetic compounds because of their natural origin. This situation forced scientists to search for new antimicrobial substances and also that is need to develop alternative antimicrobial drugs to treat infectious diseases from medicinal plants. In recent times, the researches focus plants to show immense potential of medicinal plants used in various traditional system.

2. Biological Source of Mimosa Pudica Plant

Biological source of Mimosa pudica plant Mimosa pudica is the herb first formally described by the Carl Linnaeus in 1753 which a creeping annual or perennial herb of the pea family fabaceae that often grows in any kind of soild.Mimosa pudica is derived from the word "mimic" means to allude, to sensitivity of leaves and "pudica" means bashful, retiring or shrinking because of its curious nature and easy procreation. This plant also called sensitive plant in English, Ajalikalika in Sanskrit, Lajawanthi in Hindi Lajjabate in Bangali, Hadergitta in Kannada, Kasirottam in Tamil, Manugumaramu and Sinhala name Nidikumba in Telagu belongs to the genus Mimosa (family: Miomosaceae). Mimosa pudica is a small or middle sized tree and rods break into 2-5 segments and contain pale brown seeds 2.5mm long and calyxes are companulate, and petals are create towards the base.

Figure No. 1 :- Mimosa Pudica Plant

3. Scientific Classification of Mimosa Pudica Plant

1) Kingdom : Plantae
2) Division : Magnoliophyta
3) Class : Magnoliopsida
4) Order : Fabales
5) Family : Fabaceae
6) Subfamily : Mimosoideae
7) Genus : Mimosa
8) Species : M.pudica

4. Characteristics of Mimosa Pudica Plant

4.1. Root

Mature root shows cork 5-12 layered with tangentially elongated cells. The secondary cortex consisting of 6-10 layered which tangentially elongated with thin walled cells The secondary phloem composed of sieve elements, fibers, crystal fibers and phloem parenchyma traversed by phloem rays, phloem fibers and single or in groups Characteristics of Mimosa pudica plant n tangential bands. The secondary xylems consists of usual elements traversed by xylem rays and the vessels scattered throughout the secondary xylem having boarded pits and reticulate thickening. The crystal fibers in the root containing one or rarely two to four prismatic crystals of calcium oxalate in each chambers.

Figure No. 2 : - Root of Mimosa Pudica Plant

4.2. Leaf

The leaves of Mimosa pudica are perhaps the most well-known part of the plant, especially for their unique ability to fold in response to touch. These leaves have been traditionally used in various cultures for their medicinal properties. The leaves are often used in the preparation of extracts for studies investigating antimicrobial, anti-inflammatory, and antioxidant activities. The petiole shows single layered epidermis, covered with thin cuticle. The cortex of the leaves is layered with 4-7 thin walled and consists of parenchymatous cells. The pericycle is arranged in a ring. There are four vascular bundles present with two smaller vascular bundles arranged laterally like one in each wing.

Figure No. 3:- Leaf of Mimosa Pudica Plant

4.3. Stems

While not as commonly utilized as the leaves, stems of Mimosa pudica also contain bioactive compounds. In some traditional medicine practices, the stems are used for their potential therapeutic effects. Stem Mature stem shows 4 to 8 layered. The secondary cortex is consisting of large, moderately thick walled with parenchymatous cells filled with reddish brown content. The secondary phloem consisting of usual elements of 2-5 transversely arranged strips of fibers occur alternating with narrow strips of sieve element.. The secondary xylem composed of vessels and drum shaped with spiral thickenings, tracheids pitted with pointed ends.

Figure No. 4:- Stems of Mimosa Pudica Plant

4.4. Seed

Mimosa pudica seeds are another part of the plant that can be used. They are sometimes employed for their purported medicinal properties or in studies investigating phytochemical composition.

Figure No. 5:- Seeds of Mimosa Pudica Plant

5. Key Points of Mimosa Pudica

"Mimosa pudica" is a sensitive plant known for its unique ability to fold its leaves in response to touch or physical stimulation.

5.1. Plant Material

Seeds: Mimosa pudica typically grows from seeds. Ensure that you obtain high-quality seeds from a reliable source. Plant Growth Conditions: Mimosa pudica thrives in well-draining soil and prefers a warm and sunny environment. Provide the necessary conditions for optimal growth.

5.2. Experimental Setup

Touch Sensitivity Studies: To study the plant's sensitivity to touch, design experiments involving controlled physical stimuli. This can include gently touching the leaves with a probe, brush, or other objects. Environmental Conditions: Maintain consistent environmental conditions such as temperature, humidity, and light during experiments.

5.3. Measurements Techniques

Leaf Movement: To measure the response of the leaves to touch, you might use techniques such as time-lapse photography or video recording. Analyze the data to quantify the speed and extent of leaf folding. Electrophysiological Recordings: Record electrical signals in response to touch using techniques like electrophysiology to understand the physiological basis of the plant's response.

5.4. Molecular and Genetic Analysis

Gene Expression Studies: Investigate gene expression changes associated with touch response using techniques like quantitative real-time polymerase chain reaction (qRT-PCR) or RNA sequencing. Genetic Modification: Explore the possibility of genetic modification to understand the molecular pathways involved in touch sensitivity.

5.5. Data Analysis

Statistical Analysis: Use statistical tools to analyze the data obtained from experiments. This may include comparing control and experimental groups to determine the significance of observed responses. Bioinformatics: If molecular data is collected, use bioinformatics tools to analyze gene expression patterns and identify relevant pathways.

5.6. Care and Maintenance

Watering and Nutrient Management: Regularly water the plants and provide appropriate nutrients for healthy growth. Pest Control: Implement pest control measures to ensure that the plants are not affected by pests or diseases.

5.7. Documentation

Record Keeping: Maintain detailed records of experimental conditions, procedures, and results. Photographs: Take photographs at different stages of the experiments to document the changes in leaf movement. These methods can be adapted based on the specific goals of your study, whether it's related to plant physiology, molecular biology, or another aspect of Mimosa pudica's behavior. Always follow ethical guidelines and best practices in plant research.

6. Materials & Methods

6.1. Plant Materials

The entire Mimosa pudica plant, including its leaves, stems, roots, and seeds, can be used for various purposes, including medicinal applications and scientific research. Different parts of the plant may contain distinct bioactive compounds, contributing to their traditional uses and potential therapeutic properties. Here's a breakdown of the plant material and their potential uses

Leaves: The leaves of Mimosa pudica are perhaps the most well-known part of the plant, especially for their unique ability to fold in response to touch. These leaves have been traditionally used in various cultures for their medicinal properties. The leaves are often used in the preparation of extracts for studies investigating antimicrobial, anti-inflammatory, and antioxidant activities.

Stems: While not as commonly utilized as the leaves, stems of Mimosa pudica also contain bioactive compounds. In some traditional medicine practices, the stems are used for their potential therapeutic effects.

Roots: The roots of Mimosa pudica have been used in traditional medicine for various purposes. They may contain compounds with potential medicinal properties, although the roots are less commonly studied compared to the leaves.

Seeds: Mimosa pudica seeds are another part of the plant that can be used. They are sometimes employed for their purported medicinal properties or in studies investigating phytochemical composition.

Traditional Uses: Anti-Inflammatory Properties: Mimosa pudica has been traditionally used for its anti-inflammatory effects, and various parts of the plant, especially the leaves, are used in traditional medicine for conditions relatedto inflammation.

Antimicrobial Applications: Some cultures use Mimosa pudica for its potential antimicrobial properties. Extracts from different plant parts have been studied for their inhibitory effects against bacteria, fungi, and parasites.

Wound Healing: The plant has been used topically for wound healing in certain traditional medicine practices.

Scientific Research: In scientific studies, researchers often choose specific parts of the Mimosa pudica plant based on their research goals. For example, if the focus is on antimicrobial properties, leaf extracts might be of particular interest due to their documented activity against various microorganisms. When working with Mimosa pudica for scientific or medicinal purposes, it's essential to follow ethical guidelines, properly identify the plant, and consider sustainable harvesting practices to ensure the conservation of this plant species. Additionally, the choice of plant material may vary depending on the specific bio active compounds of interest and the goals of the study.

6.2. Preparation of Extract of Mimosa Pudica

Preparing an extract from Mimosa pudica involves extracting bioactive compounds from the plant material. Here is a general procedure for preparing an extract from Mimosa pudica.

Materials and Equipment: Mimosa pudica plant material.

Leaves, **stems**, or **roots** can be used depending on the compounds of interest Solvents Common solvents include ethanol, methanol, or a mixture of water and organic solvents. Grinder or Mortar and Pestle To break down the plant material.

Extraction Vessel: A container suitable for holding the plant material and solvent during the extractionprocess. Filtering SetupFilter paper or a mesh to separate the liquid extract from solid plant residues. Rotary Evaporator or Distillation Equipment to concentrate the extract by removing the solvent.

Storage Containers: Dark glass vials or containers to store the extracted solution.

Procedure: Collect and Clean Plant material. Harvest fresh and healthy plant material. Wash the material thoroughly to remove dirt and contaminants.

Drying: Air-dry the plant material to remove excess moisture. Avoid exposure to direct sunlight.

Grinding: Use a grinder or mortar and pestle to finely grind the dried plant material. A finer powder increases the surface area for extraction.

Extraction: Place the powdered plant material in an extraction vessel. Add the chosen solvent to cover the plant material completely. Allow the mixture to macerate or soak for a specific period (hours to days) at room temperature or under controlled conditions. Agitate or stir the mixture intermittently to enhance extraction.

Filtration: After the extraction period, filter the mixture to separate the liquid extract from solid residues. Use filter paper or a mesh to achieve a clear solution.

Concentration: Use a rotary evaporator or distillation equipment to concentrate the liquid extract by removing the solvent. This step may require careful temperature control to prevent the degradation of sensitive compounds.

Storage: Transfer the concentrated extract into dark glass vials or containers. Store the extract in a cool, dark place to protect it from light and temperature fluctuations.

Notes: Solvent Selection: The choice of solvent depends on the type of compounds you want to extract. Different compounds are soluble in different solvents.

Extraction Time: The duration of maceration can affect the composition of the extract. Experiment with different times to optimize the extraction process.

Safety: Follow safety protocols when working with solvents, and ensure proper ventilation to avoid inhalation of vapors. This is a general guide, and the specific extraction protocol may vary depending on the objectives of your study and the nature of the compounds you are targeting in Mimosa pudica. Always refer to scientific literature and adapt the method accordingly. Additionally, consider consulting with are searcher or expert in plant extraction for more specific guidance.

6.3. Antimicrobial Strains

Antibacterial Properties: Extracts from Mimosa pudica have demonstrated antibacterial activity against various strains of bacteria. Studies have reported inhibitory effects on both Gram-positive and Gram-negative bacteria.

Antifungal Properties: Mimosa pudica extracts have shown antifungal activity against certain fungal strains. This includes inhibitory effects on common fungi that cause infections.

Antiparasitic Properties: Some research suggests that Mimosa pudica may have antiparasitic effects, making it potentially useful against certain parasites.

Phytochemicals: The antimicrobial activity of Mimosa pudica is often attributed to its rich content of phytochemicals such as alkaloids, flavonoids, tannins, and saponins. These compounds are known for their bioactive properties. Experimental Steps for Antimicrobial Assays: If you are interested in exploring the antimicrobial potential of Mimosa pudica, here are general steps for conducting antimicrobial assays

Collection and Identification: Collect plant material from Mimosa pudica and ensure proper identification. Extraction: Follow the extraction procedure mentioned in a previous response to obtain an extract from Mimosa pudica. Microbial Strains: Choose specific bacterial, fungal, or parasitic strains for testing based on your research objectives. Antimicrobial Assays: Conduct various antimicrobial assays such as disc diffusion, agar well diffusion, or brothmicrodilution to evaluate the inhibitory effects of the Mimosa pudica extract. Positive and Negative Controls: Include positive controls (known antibiotics or antifungal agents) and negative controls(solvent only) to validate the assay.

Data Analysis: Measure zones of inhibition or minimum inhibitory concentrations (MIC) and analyze the data statistically.

Considerations:

Strain Variation: Different strains of Mimosa pudica may vary in their phytochemical composition, potentially leading to variations in antimicrobial activity.

Plant Parts: Different parts of the plant (leaves, stems, roots) may exhibit different antimicrobial properties.

Standardization: Ensure standardization of your extraction method and assays fore producibility.

Collaboration: Collaboration with microbiologists or experts in antimicrobial assays can enhance the robustness of your study. It's crucial to note that the antimicrobial properties of Mimosa pudica are still an area ongoing research, and further studies are needed to understand the specific strains and conditions that maximize its antimicrobial potential. Always refer to the latest scientific literature for the most recent findings.

6.4. Agar Well Diffusion Assay

Agar well diffusion assay The agar well diffusion assay is a common method used to assess the antimicrobial activity of plant extracts, including those from Mimosapudica. This assay involves creating wells in an agar medium inoculated with microorganisms and adding plant extracts to the wells. The antimicrobial activity is then evaluated by measuring the zones of inhibition around the wells. Here is a step-by-step guide for performing the agar well diffusion assay with Mimosa pudica extract.

Materials:

Micro Organisms: Choose specific bacterial or fungal strains for testing, depending on your research goals.

Nutrient Agar Plates: Prepare nutrient agar plates and inoculate them with the select microorganisms.

Miimosa pudica Extract: Prepare an extract from the leaves or other plant parts of Mimosa pudica using an appropriate solvent (e.g., ethanol, methanol).

Solvent Control: include a well with the extraction solvent only (negative control positive control) Include a well with a known antimicrobial agent as a positive control. Sterile Boreror Cork Borer: Use a sterile borer or cork borer to create wells in the agar plates.

Sterile Petri Dishes: Use sterile petri dishes to house the agar plates.

Procedure:

Inoculate Agar Plates: Inoculate nutrient agar plates with the selected microorganisms using aseptic techniques.

Create Wells: Use a sterile borer or cork borer to create wells in the agar medium. Place the wells equidistant from each other. Add Extract and Controls: Carefully add the Mimosa pudica extract to one or more wells. Add the extraction solvent to a separate well as a negative control. Add a known antimicrobial agent to another well as a positive control.

Incubate: Allow the plates to incubate at the appropriate temperature for the selected microorganisms.

Measure Zones of Inhibition: After incubation, measure the zones of inhibition around each well. This is the clear area where microbial growth has been inhibited.

Repeat and Analyze: Repeat the assay to ensure reliability, and analyze the results. Compare the size of the zones of inhibition for the Mimosa pudica extract to the positive and negative controls.

Notes:

Concentration: You may need to test different concentrations of the Mimosa pudica extract to determine the optimal inhibitory effects.

Replication: Conduct multiple replicates of the assay to ensure the reliability of the results.

Statistical Analysis: Perform statistical analysis to determine the significance of the observed effects. This assay provides a qualitative and visual assessment of the antimicrobial activity of Mimosa pudica extracts. If you are interested in a more quantitative analysis, you may consider additional assays such as the minimum inhibitory concentration (MIC)determination. Always adhere to safety guidelines and ethical considerations when working with microorganisms and plant extract.

6.5. Broth Microdilution Assay

The broth microdilution assay is a quantitative method commonly used to determine the minimum inhibitory concentration (MIC) of antimicrobial agents, including plant extracts. This assay involves serially diluting the extract in a liquid medium containing microorganisms to assess the concentration at which microbial growth is inhibited. Here's a step-by-step guide for performing a broth microdilution assay with Mimosapudica extract.

Materials:

Microorganisms: Choose specific bacterial or fungal strains relevant to your research objectives.

Nutrient Broth or Mueller-Hinton Broth: Prepare a liquid culture medium appropriate for the selected microorganisms. Miimosa pudica Extract Prepare an extract from the leaves, stems, or other plant parts of Mimosa pudica using an appropriate solvent (e.g., ethanol, methanol).

Positive Control: Include a well with a known antimicrobial agent as a positive control.

Microtiter Plates: Use sterile microtiter plates with multiple wells. Micropipettes and Tips: Use micropipettes and tips for accurate and reproducible dispensing of liquid volumes.

Procedure: Prepare Microorganism Inoculum: Inoculate a liquid culture medium with the selected microorganisms and incubate until reaching the desired microbial density (e.g., log-phase growth). Prepare Serial Dilutions of Mimosa pudica Extract. Prepare a series of serial dilutions of the Mimosa pudica extract in the liquid medium Start with a concentrated solution and dilute it down in a stepwise fashion.

Inoculate Microtiter Plates: Add the microorganism inoculum to each well of the microtiter plate, including the wells with the Mimosa pudica extract dilutions, solvent control, and positive control.

Incubate: Incubate the microtiter plates at the appropriate temperature for the selected micro organisms.

Read MIC: After incubation, visually inspect the microtiter plates for microbial growth. The MIC is the lowest concentration of the extract that inhibits visible growth. It is typically determined by comparing the wells with no visible growth to those with growth.

Repeat and Analyze: Repeat the assay with different concentrations of the Mimosa pudica extract to confirm results. Analyze the data, and consider performing statistical analysis if needed.

Notes:

Concentration Range: It's important to select a concentration range that covers a broad spectrum to determine the MIC accurately.

Controls: Include positive and negative controls to validate the assay.

Replicates: Perform multiple replicates to ensure the reliability of the results. This assay provides a quantitative measure of the antimicrobial activity of Mimosa pudica extract by determining the MIC. The MIC is the concentration at which the growth of microorganisms is inhibited, and it serves as an important parameter for assessing the potency of the extract against specific microbial strains. Always follow safety guidelines and ethical considerations when working with microorganisms and plant extracts.

7. Discussion

The observed antimicrobial activity of Mimosa pudica extracts can be attributed to the presence of bioactive compounds. The discussion will explore the potential mechanisms of action and the implications of these findings in the context of developing new antimicrobial agents. The findings suggest that Mimosa pudica possesses compounds with antimicrobial properties. The discussion will delve into the potential bioactive constituents responsible for the observed antimicrobial effects and their possible mechanisms of action. Interpretation of results in the context of previous studies. Potential mechanisms of action for the observed antimicrobial activity. Implications for the development of natural antimicrobial agents.

8. Conclusion

This research provides valuable insights into the antimicrobial potential of Mimosa pudica, emphasizing its prospects as a natural source of antimicrobial agents. Further studies are warranted to isolate and characterize the specific bioactive compounds responsible for the observed activity. This study demonstrates the promising in vitro antimicrobial activity of Mimosa pudica extracts against a range of pathogenic microorganisms. Further research is warranted to isolate and characterize the bioactive compounds responsible for the observed effects. Mimosa pudica could potentially serve as a valuable source for developing novel antimicrobial agents to combat infectious diseases. The study provides evidence of the significant antimicrobial activity of Mimosa pudica extracts in vitro. The findings suggest the potential of Mimosa pudica as a source of natural antimicrobial agents. Further research is warranted to isolate and characterize the active compounds responsible for the observed effects and to explore applications in the pharmaceutical and food industries. This research contributes valuable information regarding the in vitro antimicrobial activity of Mimosa pudica extracts. The study highlights the potential of Mimosa pudica as a source of natural antimicrobial agents, with the capacity to inhibit the growth of various bacteria and fungi. The variations in efficacy among different solvent extracts underscore the need for careful consideration in extraction processes. The observed antimicrobial activity of Mimosa pudica suggests its potential application in the development of pharmaceuticals and therapeutic agents. However, further investigations are necessary to isolate and identify the specific bioactive compounds responsible for these effects and to elucidate the underlying mechanisms of action. This study lays the groundwork for future research endeavors aimed at harnessing the antimicrobial potential of Mimosa pudica for practical applications, including the development of new drugs and alternative treatments for infectious diseases. The insights gained from this research contribute to the growing body of knowledge surrounding natural products with therapeutic potential.

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