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PHYTOCOGNOSTICAL, ANALYTICAL STUDIES, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF SHOOTS AND ROOTS OF *MIMOSA PUDICA LINN.*

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ABSTRACT

The plant known as Mimosa pudica is found in Brazil and India. Across the tropics and subtropics of Asia, Africa, South America, North America, and Australia, the genus Mimosa is distributed. This genus has long been used to treat a wide range of conditions, including smallpox, hepatitis, tumors, HIV, ulcers, ringworm, asthma, leprosy, jaundice, diarrhea, fever, toothaches, wound healing, and skin and urinary issues. The Soxhlet extraction process uses water, methanol, chloroform, petroleum ether, and coarsely dried shoots and roots as the solvents. Within vacuum desiccators, the extract was concentrated. The transverse section histological analysis of the mimosa pudica root and shoot is carried out. The results of a quantitative physical examination were recorded for the shoot and root, including the total ash value, acid-insoluble ash, water-soluble ash, water-insoluble ash, water-soluble extract, alcohol-soluble extract, and ether-soluble extract. Alkaloids, polysaccharides, glycosides, and phytosterols have been identified by preliminary chemical investigation in MSME and MRME. MSME, MRME, MSCE, MRCE, MRWE, and MSWE are utilized as test compounds and DMSO is used as a blank when the gentamycin medicine was employed as a standard to determine the antibacterial activity. The disc diffusion method is used to measure the in-vitro antibacterial activity of the gram-positive bacteria Bacillus. When compared to the conventional gentamycin, MSME and MRME showed good antibacterial action against gram (+) ve bacterium bacillus. Clotrimazole is utilized as a standard, DMSO is used as a blank, and MSME, MSCE, MSWE, MRME, and MRWE are employed as test compounds in

the case of antifungal activity. The disc diffusion method is used to measure the in-vitro antifungal activity of Aspergillus niger. Aspergillus niger does not exhibit any antifungal action from any of the test chemicals.

KEYWORDS: Phytochemical screening, Disc diffusion method, Antibacterial, Anti-fungal.

1. INTRODUCTION

A creeping herbaceous ornamental plant in the Mimosaceae family is called Mimosa pudica L. In ethnomedicine, it is used to treat or prevent several diseases, including urinary tract infections, diabetes, alopecia, cancer, and diarrhea.[1] Species that fold their leaves in reaction to contact include M. pudica and M. pigra. Flowers can be in clusters, white, or globular pink. The fruit walls are compressed in the center of the seeds, and the fruits are brittle.[2] Researchers from all around the world are drawn to Mimosa pudica because of its pharmacological properties, which include anti-diabetic, antitoxin, antihepatotoxin, antioxidant, and wound-healing properties. It is said to include tannis, glycoside, alkaloids, and flavonoids. It is used to treat wound healing, blood coagulation, sexual weakness, and kapha and pitta suppression.[3] Mimosa pudica, sometimes referred to as the touch-me-not, humble, or sensitive plant, is a highly well-liked plant worldwide because of its delicate character, which many people find enjoyable.[4] In Ayurveda, M. pudica, or Lajjalu, is used extensively as an antidepressant and an asthma reliever to treat a wide range of illnesses. Additionally, epidemiological research has demonstrated that M. pudica includes metabolites with anticancer and antidiabetic effects, such as phenols and flavonoids. Additionally, studies have shown that various portions of this plant are frequently utilized to cure a variety of illnesses.[5] The shrubby Mimosa pudica (Mimosaceae) has lilac pinkish axillary flower heads, spinout stipules, glandular hairs, bipinnate leaves, and campanulate calyxes. The stems are well-branched and upright.[6] Numerous ethno-medical uses of Mimosa pudica Linn have been observed in traditional medical systems.[7] Mimosa pudica is a South American native that is a tropical weed in the legume family. The herb has long been used in traditional medicine to treat wound healing, diarrhea, nasal problems, and urogenital disorders.[8] The herb Mimosa pudica (Linn.) is a perennial or annual swarmer. It has been recorded in Ayurveda as lajjalu. It is also known by several other names, including Chui Mui, lajwanti, sleeping grass, unstable plant, humble plant, hesitant plant, disgrace plant, resting grass, contactmenot, namaskari in Sanskrit, and others.[9] Due to its well-known sedative, emetic, and tonic qualities, M. pudica has been traditionally used to treat a wide range of illnesses,

including urogenital infections, tumors, alopecia, diarrhea, and dysentery. Studies on the phytochemistry of M. pudica have identified alkaloids, sterols, terpenoids, tannins, fatty acids, and mimosine, a non-protein amino acid. An extract of its leaves has been shown to have a chemical similar to adrenaline. A phytochemical study of the root of M. pudica revealed the presence of ascorbic acid, crocetin, D-glucuronic acid, linoleic acid, linolenic acid, palmitic and stearic acids, mimosine, D-xylose, and b-sitosterols. It was reported that seeds produced sitosterol. In the Unani medical system, roots are alternative, resolvent, and helpful in treating bilious fevers, piles, jaundice, leprosy, and other conditions caused by bile and blood impurities. The lethality of the venom of the monocled cobra (Naja kaouthia) has been significantly reduced by aqueous extracts of the plant's roots. It seems to reduce cobra venom's myotoxicity and enzyme activity.[10] It is referred to as "sparshaat sankochataam yaati punashcha prasruta bhavet" in Ayurveda; it is a plant that folds itself when touched and then spreads its leaves once again after some time. Lajjalu possesses tikta and kashaya rasa, or an astringent and bitter taste, according to Ayurveda.[11] The mimosa pudica plant grows throughout several parts of India, including Tamil Nadu, Kerala, Orissa, Karnataka, Andhra Pradesh, and Telangana. There is proof that this plant exhibits thigmonasty. Numerous biological qualities are present in the plant, including antibacterial, antidiabetic, anticonvulsant, antivenom, anti-inflammatory, anticancer, antifungal, antinociceptive, and antiulcer activity. It also exhibits antihistamine, wound healing, diuretic, antioxidant, and antifertility effects. The herb has long been used in traditional medicine to treat wound healing, diarrhea, nasal problems, and urogenital disorders.[12] A component of the traditional medical systems of America, Africa, Korea, China, and India is Mimosa pudica Linn. For millennia, it has been utilized in traditional medicine to treat a variety of illnesses, including dyspepsia, fever, diabetes, constipation, jaundice, ulcers, and biliousness. It is a crucial component of many different kinds of herbal remedies.[13] The foundation of herbal medicine is the notion that plants have inherent chemicals that can support health and healing. The plant Mimosa pudica means "to point" and "to be shy." Samberg used the sensitivity of Mimosa pudica in an attempt to connect the neurological capabilities of plants with mammalian systems. M. Pudica seeds are extruded into glucuronoxylan polysaccharide, a hydrogelable substance that is used to target and/or postpone the release of certain drugs.[14] Scientists have realized that, given the current situation of multiple medicine resistance, screening plants is essential to finding novel medications with therapeutic efficacy. Mimosa pudica's many ethnobotanical applications have been researched. Lajjalu, as it is known in Ayurveda, is a creeping herb that can be perennial or annual. Because of its pharmacological

qualities, which include antidiabetic, antitoxin, antihepatotoxin, antioxidant, and wound-healing capabilities, it is one of the most sought-after herbs. The Unani and Ayurvedic medical systems both make extensive reference to the plant. The various portions of mimosa have been used historically—and currently in some regions of the world—to treat a variety of ailments and discomforts. This plant's decoction of roots is used to treat toothaches. Mimosa pudica is said to halt the bleeding and hasten the wound's healing process. It is mostly used in herbal treatments for problems related to gynecology.[15] Mimosa pudica has long been used in traditional medicine to treat a variety of ailments. This plant, which is also known as "touch me not plant" in our country, is cultivated during the rainy season. It should go by different names in other languages, such as "sensitive plant" in English, "lazonthi" in Hindi, and "tottasiningi" in Tamil. Inflammation, diabetes, fever, piles, and other conditions are treated using remote populations.[16] Mimosa pudica has been used traditionally in folk medicine to treat a wide range of conditions, including infections, rheumatoid arthritis, convulsions, anxiety, depression, bleeding disorders, muscular discomfort, asthma, and snake bites.[17] The mimosa plant is a shrub that grows quickly and can be classified as either an annual or perennial herb. Because of the unusual movement of its touch-sensitive leaflets, this plant is interesting to look at. When touched by a hand or any other item, living or dead, its fem-like leaves shut up and hang downward. Because of the unique qualities of its leaves, mimosa is valued as a plant with high ornamental value. These leaves become open quite quickly after the stimulus is removed. Mimosine, an alkaloid, has been found in the plant's stems and leaves. Mucilage and tannins are also present in the leaves. Turgorins are also present in the plants. M. pudica is considered an astringent, diuretic, and antispasmodic. Fistula and piles are treated using leaves and roots. Hydrocele is treated using leaf paste. Sinuses are dressed with cotton that has been soaked with leaf juice. Additionally helpful as a blood purifier, plants can be utilized to heal gum sores. It is also used to treat children's convulsions.[18] It is referred to as "sparshaat sankochataam yaati punashcha prasruta bhavet" in Ayurveda; it is a plant that folds itself when touched and then spreads its leaves once again after some time. Lajjalu has an astringent and bitter flavor, or tikta and kashaya rasa, according to Ayurveda.It balances kapha and pitta and has a cold (sheetha) quality. According to reports, it can be used to treat bleeding piles, diarrhea (athisaara), amoebic dysentery (raktaatisaara), and stop bleeding.[19] Because of its cytotoxic, anti-diarrheal, and anti-hyperglycemic qualities, Mimosa pudica Lin. has been utilized traditionally.[20] The present study focuses on the Phytocognostical studies and Antimicrobial activities of methanol. Chloroform, water extracts of Shoots and Roots of Mimosa pudica Linn.

2. METHODOLOGY

2.1 Histological studies

Transverse section of stem

Epidermal cells

There are several layered guad rectangular cells with smooth, thick cuticles.

Cortex

Palisade cells

Palisade cells with chloroplasts are organized loosely.

Lignified fibres

Below the stiff, there is a cluster of lignified fibers.

Xylem

Vascular, tracheids, fibrotracheids, and parenchyma are all well-developed.

Pith

Lignified, large, polygonal parenchyma with thin walls and intracellular space.

The transverse section of Shoot

Cork

It is possible to discern stratified cells in shifting bands of lignified cells.

Phelloderm

A few layers of isodermic parenchyma cells that are tangentially extended. Crystals of calcium oxalate and starch grains are present in a few cells.

Secondary xylem (lignified)

The secondary xylem contains xylem vessels, xylem fibers, and xylem parenchyma.

Secondary phloem

Crystals of calcium oxalate are seen in tubes, cells containing starch grains, and phloem parenchyma.

Pith

There's a tiny pith at the Centre.

2.2 Determination of ash values

After being coarsely ground, the air-dried shoot and root of "mimosa pudica" were examined. The following ash values' percentages were computed using the air-dried excavation as a reference.

Ash is intended to be the residue that remains after the medication is burned. The residue that comes from the inorganic components of the plant could be intended to be physiological ash. The ratio fluctuates within predetermined bounds depending on the types of soil, dust, and sand. Mineral impurities or the use of other medications can also change the ratio.

The different types of ash value are the following

A. Total ash value

B. Alcohol insoluble ash value

C. Water soluble ash value

Determination of Total Ash Value

Weigh a sintered glass crucible precisely after it has cooled and been ignited. around two grams of medication in powder form. For example, place the mimosa pudica root and shoot in the dish separately and use the stand to support the crucible. Heat the crucible on low heat at first, then increase the heat until all of the carbon has burned off.

Percentage total ash value of the mimosa pudica shoot = 6.45% Percentage total ash value of the mimosa pudica root = 7.15%

B. Alcohol insoluble ash value

Using a Bunsen burner, incinerate the precisely weighed powder (2 grams) of the root and shoot it separately in a silicon crucible. Pour the ash from the plate into a 100ml beaker and wash it with 25ml of alcohol (methanol). Put over a hob with a wire mesh and boil for five minutes. Pour residue through an ash-free filter paper and heat it to 100 degrees Celsius.

Place the residue and dried filter paper into a crucible that has been previously weighed. Heat the mixture gradually until the vapors stop evolving, then more intensely until all of the carbon has been eliminated. Weigh after cooling. Calculating the percentage of alcohol-insoluble ash can be done based on this weight difference.

Percentage of alcohol insoluble ash value of mimosa pudica shoot = 0.75%

Percentage of alcohol insoluble ash value of mimosa pudica root = 0.55%

C. Water soluble ash value

Using a Bunsen burner and 25 milliliters of distilled water, incinerate the precisely weighed powder (2 grams) of the shoot and root separately in a silica crucible. Then, transfer the ash from the dish into a 100-millilitre beaker. Place over a hob on a wire mesh and boil for five minutes. Use an ashless filter paper, give the residue two thorough washes in hot water, and then dry the residue and filter paper at 100˚C in the oven.

Place the residue and dry filter paper in a crucible that has been previously weighed. Heat the mixture slowly at first until the vapors stop evolving, and then more vigorously until the carbon has been eliminated. Weigh after cooling. It is possible to compute the percentage of water-soluble ash from these weight differences.

Percentage of water-soluble ash value of mimosa pudica shoot = 0.45%

Percentage of water-insoluble and value of mimosa pudica root = 0.35%

2.3 Determination of extractive value

A. Water soluble extractive value

B. Alcohol soluble extractive value

C. Ether soluble extractive value

Water soluble extractive value

Medications that contain water-soluble active components of crude medications, such as sugars, tannins, plant acids, mucilage, etc., are treated with a water-soluble extractive. It is applied to both the shoot and root extracts.

Water soluble extractive value of the 'mimosa pudica shoot'=11.5%

Water soluble extractive value of the 'mimosa pudica root' =8.5%

Alcohol soluble extractive value

Methanol is employed in the extraction of alcohol-soluble extractives, which are most appropriate for figuring out the drug's resin content. Take two grams of the powdered medication, taking the extracts of the stalk and root separately. Then add the methanol, give it a good shake, filter, collect the filtrate, let it evaporate, and weigh each ingredient separately.

Alcohol soluble extractive value of the mimosa pudica shoot = 10%

Alcohol soluble extractive value of the mimosa pudica root =7.5%

Chloroform Soluble Extractive Value

Drugs that comprise additional soluble active components of crude oil, such as resin, fixed oil, or colouring matter, can be treated with chloroform soluble extractive value. For the chloroform soluble extractive, it was applied to both the shoot and the root.

Chloroform soluble extractive value of the mimosa pudica shoot =2.5%

Chloroform soluble extractive value of the mimosa pudica root=1.5%

2.4 Preliminary chemical studies

Extractive process

At 4 p.m., the "mimosa pudica" plant was picked in the inner Kanyakumari district of Tamil Nadu, close to Mathur. For extraction, the young root and shoot are utilized. The shoots were extracted for five hours using petroleum ether, twelve hours using chloroform, and twenty-four hours using methanol and water. Water, methanol, and chloroform are used as a solvent for hot continuous extraction using Soxhlet equipment and petroleum ether are not included in the root extraction. The *mimosa pudica* plant was present in the fig 1.

Procedure

A separate 250 gm portion of the shoot and root were coarsely ground and extracted using petroleum ether, chloroform, methanol, and water for the shoot. The extracts from root chloroform, methanol, and water were separated and vacuum-desiccated to concentrate them. After that, the concentrated extract was kept in a vacuum desiccator to dry. The weight of the extract was used to compute the % yield for the shoot and root, which are shown in Tables 2 and 3, respectively.

2.5 Analytical studies

Thin Layer Chromatography

Principle

The material that is more soluble in the solvent will flow with it, whereas the material that is more adsorbent in the silica gel will be adsorbed by the silica gel.

Preparation of thin-layer chromatography

TLC plates were prepared using an amorphous porous material and 60g of silica obtained from Mesik India Ltd. Glass plates measuring 10 x 20 cm were used. The absorbent was mixed in a mortar until it reached a smooth consistency with the necessary amount of water. The mixture was then quickly spread over the chromatographic glass plate, and after a further

30 minutes, the plate was carefully transferred to a holder and dried at 100 ˚C to 120 ˚C for an hour to activate the adsorbent. Finally, the plates were cooled and stored in a desiccator over silica gel. The spot size was approximately 0.3 cm, and the thickness of the moist thin layer was approximately 2.5 ml volume of extract applied with the help of a capillary tube. The extract was applied multiple times, allowing the spots to dry completely before adding another volume of solution to the same location. After letting the solvent evaporate, the plate was moved to a tank that had already been growing. About thirty minutes before the plate was inserted, this preparation was completed. To keep the atmosphere in the tank saturated with solvent vapor, the filter paper was dipped into the developing solvent and placed within the tank. This is done so that the extract goes more quickly down the edge of the plate than it does in the middle. Then gave the solvent time to rise at a distance of ten to fifteen centimeters. After taking the plates out of the developing tank, marking the solvent front, and letting them dry with heat, the separated areas were found using ultraviolet light. After that, the plates were sprayed with a spray reagent solution and allowed to dry. Various chromatographic solvent systems were tested for various fractions. The values of Rf were computed. The Rf value is the ratio of the distance traveled by the solute front to the distance traveled by the solvent front. Rf value = Distance traveled by solute / Distance traveled by solvent

Chromatographic Solvent System Tried For Methanolic Shoot Extract

Detection: UV

Spray reagent used: Dragendorff's reagent

It was found that the chloroform: methanol (85:15) solvent system was the most successful among the several chromatographic solvent systems tested for methanolic shoot extract, as it effectively separated seven spots. The trial result is presented in Table 4.

Chromatographic Solvent System Tried For Methanolic Root Extract

Detection: UV

Spray reagent used: Dragendorff's reagent

Chloroform: methanolic (85:15) was shown to be an extremely successful solvent system among the several chromatographic solvent systems examined for methanolic root extract, as effective separation of five spots was obtained. The trial result is presented in Table 5.

Thin Layer Chromatographic Data Analysis for Methanolic Shoot Extract

The methanolic shoot extract thin layer chromatogram displayed greenish-brown and greenish-black color spots in the Rf range of 0.5333 to 0.9000. After the chromatogram was first seen in the ultraviolet light, Dragendorff's reagent was sprayed on it. The result is presented in Table 6.

Adsorbent : Silica gel 60g
Solvent system : Chloroform: methanol (85:15)
Spray reagent : Dragendorff's reagent

Thin Layer chromatographic data analysis for Methanolic Root Extract

The Rf range of the brown and yellow color spots in the thin layer chromatogram for methanolic root extract was 0.5357 to 0.8000. The chromatogram was first observed under UV light and then Dragendorff's reagents were sprayed on it. The result is presented in Table 7.

Adsorbent : Silica gel 60gm
Solvent system : Chloroform: methanol (85:15)
Spray reagent : Dragendorff's reagent

Thin Layer Chromatographic Data Analysis for Isolated Compound (MSME)

The isolated compound's thin layer chromatogram displayed a green color spot with an Rf value of 0.8093. Under UV light, the chromatogram was visible. The result is presented in Table 8.

Adsorbent : Silica gel 60g
Solvent system : Chloroform: methanol (85:15)
Detection : UV

Thin Layer Chromatographic Data Analysis for Isolated Compound (MRME)

The isolated compound's thin layer chromatogram displayed a light brown-green spot with an Rf value of 0.5744. Under UV light, the chromatogram was visible. The chromatogram was visualized under UV. The result is presented in Table 9.

Isolation of crystal

The use of activated charcoal separates the crystal from the other active ingredients in MSCE. Crystals with a cuboidal form and no color were effectively separated. The MSCE-isolated crystal was present in Fig 2.

2.6 Qualitative Chemical Evaluation of Mimosa Pudica Root & Shoot

MSME and MRME were subjected to qualitative tests for the identification of various plant constituents. The result is presented in Table 11.

Detection of carbohydrates

Dissolve a minimum amount of extracts in 5 ml of distilled water and filter. The filtrate was subjected to Molish's test to detect the presence of carbohydrates.

A. Molish's test

After treating the filtrate with two to three drops of 1% alcoholic α-naphthol, 2 millilitres of concentrated sulphuric acid were poured along the test tube's sides. A ring of violet color appeared where the two liquids met. Regarding the presence of carbohydrates, it had a favourable reaction to the MSME and MRME extracts.

B. Fehling's test

A brick-red hue is produced when the drug extract is heated with Fehling solutions A and B.

C. Tollen's test

When Tollen's reagent and drug extract are heated, the test tube's sides take on a silver reflection.

D. Barfoed's test

When a drug extract was heated with Barfoed's reagent, it turned crimson.

Test for Glycoside

A water bath was used to hydrolyze a small amount of each extract with hydrochloric acid for two hours. The hydrolysate was then tested for the presence of various glycosides using Borntrager's and Legal's tests.

Legal's test

To the extract of hydrolysate add a few drops of sodium nitroprusside solution, and 1ml of sodium hydroxide was added to turn it alkaline. The methanolic and chloroform extracts had a pink to yellow appearance, which indicated the presence of glycoside in MRME and MSME.

Bontrager's test

After applying chloroform to the hydrolysate extract, the layer of chloroform was separated. An equal amount of diluted ammonia solution was added to this. The colors pink were seen in MRME and MSME.

Modified Borntrager's test

Five millilitres of ferric chloride solution and a few milliliters of diluted hydrochloric acid were added to the boiling methanolic extract. After being chilled, the contents are mixed with an organic solvent. After separating the organic layer, an equivalent volume of ammoniacal solution was added. The layer of ammonical revealed a pink color. To break the c-c bond of the glycosides in this assay, ferric chloride was introduced. which is more potent than glycosides' C=O linkage.

Detection of Alkaloids

A small amount of the extracts were processed individually and filtered after being exposed to a few drops of diluted hydrochloric acid. Several alkaloid reagents, including Mayer's reagent, were applied to the filtrate. The three reagents are Hager's, Wagner's, and Dragendorff's.

Wagner's test

A few drops of Wagner's reagent were applied to 1 millilitre of the filtrate. Both extracts yielded a reddish-brown precipitate, which suggested the presence of alkaloids.

Dragenrdorff's test

A few drops of Dragendorff's reagent were added to 1 millilitre of the filtrate. Both extracts yielded an orange-red precipitate, which is indicative of the presence of alkaloids.

Mayer's test

A few drops of Mayer's reagent were applied to 1 millilitre of the filtrate. Both extracts yielded an orange-red precipitate, which is indicative of the presence of alkaloids.

Hager's test

A few drops of Hager's reagent were applied to 1 ml of the filtrate. Alkaloids are present in the MRME and MSME extracts as indicated by the yellow precipitate that is formed.

Detection of phytosterols

Five millilitres of chloroform were used to dissolve small amounts of MRME and MSME separately. The Salkowski and Liebermann-Burchard test was then used for these chloroform solutions to identify any phytosterols.

Salkowski test

A few drops of strong sulphuric acid were added to 1 millilitre of the chloroform solution that was previously made. In the lowest layer, both extracts generated a red hue. It demonstrated the phytosterols' existence.

Liebermann-Burchard test

A few drops of strong sulfuric acid and one millilitre of acetic anhydride solution were added to the above-mentioned chloroform solution. Both extracts generated a green hue, which suggested the presence of phytosterols.

Detection of saponins

The extract was diluted with 20 milliliters of distilled water and then agitated in a graduated cylinder for fifteen minutes. The fact that neither extract has a foam layer indicates that saponins are present.

Detection of fixed oil and fats

a) Two filter papers were used to separate little amounts of different extracts. There are no oil stains to indicate that there are no permanent oils present.

b) A few drops of 0.5 alcoholic potassium hydroxide and a few drops of phenolphthalein were added to different extracts. The mixture was cooked for more than one to two hours in both types of water. The lack of soap formation suggests that there are no stable fats or oils present.

Detection of Tannins

a) Test for gelatin

The extracts were separated, dissolved in a small amount of water, and then filtered. Add 1 milliliter of a 1% gelatin solution to the filter. Not a single extract produced data that were indicative of the absence of tannins.

a) Test for ferric chloride

Each extract's residue was dissolved in a separate batch of water, and then a few drops of ferric chloride solution were added. There was no bluish-black precipitate, which suggests that there were no tannins present.

Detection of proteins and amino acids

After the extracts were dissolved in a small amount of water, they underwent the Million's biuret and ninhydrin tests.

Million's test

Million's reagent was applied to the extracts that were previously produced. It generated a reddish color.

The biuret test

An equal volume of 1% copper sulfate and 5% sodium hydroxide were added to the above-prepared extracts. It generated a violet color.

Test for ninhydrin

The ninhydrin reagent was applied to the aforementioned extracts. The color turned either pink or blue. The results of the three tests mentioned above show the existence of amino acids and proteins, respectively.

Detection of flavonoids

a. Shinoda's test

A small amount of the extracts were dissolved in alcohol, and then dropwise additions of strong hydrochloric acid were added and heated to the magnesium metal.

Magenta hue was formed in MSME extracts, showing the presence of flavonoids.

b. Flavones

a) When the extracts were dissolved in sodium hydroxide solution, they did not become yellow.

b] Concentrated sulfuric acid did not give either extract an orange hue.

c. Zinc, HCL reducing test

A tiny amount of each extract was mixed with a small pinch of zinc dust. A few drops of strong hydrochloric acid were then added. After a few minutes, not all of them generated a magenta color.

d. Lead acetate solution test

Ten percent lead acetate solution was applied, drop by drop, to a small amount of each extract. The extracts did not provide any yellow precipitate, a sign that flavones or flavanones were not present.

Detection of Coumarins

After being dissolved in alcohol, the tiny amount of extracts was exposed to UV light. It doesn't emit green fluorescent light.

2.7 In-vitro antimicrobial activity

2.7.1 In-vitro antibacterial activity by disc diffusion method

Plates of nutrient agar were made aseptically to a thickness of 5.6 mm. To stop condensate from spilling onto the agar surface, the plates were allowed to harden and then turned over. Right before being inoculated, the plates were dried at 37 ˚C. The standard inoculums were deposited onto previously prepared (aseptic) plates by dipping a sterile swab into the inoculate, scraping off excess inoculate by pressing and rotating the sub firmly against the sides of the culture tube above the liquid level, and then streaking the swab all over the medium three times. Each time, the plate was rotated at an angle of 60˚C. Lastly, seal the lid and squeeze the swab around the agar surface's edge, allowing the inoculums to dry at room temperature. To enable uniform diffusion, the sterilized discs for the test medicines were placed in Petri dishes and aseptically incubated at 37±0.2 ˚C for approximately 18–24 hours after being refrigerated for one hour. The plates' average zone diameter was measured and noted. All synthesized compounds were tested for antibacterial activity. The result was present in the table 12 & 13 respectively for shoot and root extracts of *mimosa pudica*. The zone of inhibition of shoot and root extracts of mimosa pudica was present in Fig 3.

2.7.2 In-vitro antifungal activity by disc diffusion method

To avoid condensate from dripping on the agar surface, Sabouraud dextrose agar plates are produced aseptically to a thickness of 5–6 mm. The plates are then dried at 37 ˚C just before inoculation. The standardized inoculums in the plates were previously prepared by dipping a

sterile swan into the inoculums, then pressing and rotating the swab firmly against the sides of the culture tube above the liquid level and finally streaking the swab three times over the medium's surface to remove any excess inoculums. After every application, rotate the plate at a 60 ˚ angle. Lastly, circle the corners of the agar surface with the swab. Keep the lid covered and allow the inoculation to dry at room temperature. After sterilizing the discs for the test drug, they were carefully transferred to Petri dishes. Then, they were placed on solidified sabouraud agar media. The Petri dishes were aseptically incubated at 30 ˚C for a minimum of 18 to 48 hours after being refrigerated for one hour to aid in uniform diffusion. The average zone diameter of the plates was measured and recorded. All compounds were tested for antifungal activities. The results for the shoot and root extracts of mimosa pudica were present in the table 14 and 15 respectively.

3. RESULT AND DISCUSSION

The Soxhlet extraction procedure was carried out by using coarse dried shoot and root with the solvent petroleum ether chloroform, methanol, and water. The extract was concentrated in a vacuum desiccator. The histological study (transverse section of shoot and root of mimosa pudica is performed. The quantitative physical analysis of total ash value, acid insoluble ash, water soluble ash, water insoluble ash, water-soluble extract, alcohol soluble extract, and ether-soluble extract for the shoot and root and results are recorded in table no.1. Preliminary chemical analysis indicates the presence of alkaloids, carbohydrates, glycosides, and phytosterols in MSME and MRME. The TLC study performed for MSME showed a 7-spot and for MRME showed a 5-spot and isolated crystal of MSME and MRME showed a single spot when visualized in UV light at 254nm. In the case of antibacterial activity of gentamycin test drug, MSME, MRME, MSCE, MRCE, MRWE, MSWE, and blank are determined by disc diffusion method against the bacillus (gram + ve microorganism). The zone of inhibition of Standard drug gentamycin, MSME, and MRME were found to be 25 mm, 9mm, and 6mm respectively. In the case of antifungal activity of the standard drug clotrimazole, test drug MSME, MSCE, MSWE, MRME, MRWE, and blank are determined by the disc diffusion method against the microorganism Aspergillus Niger. Except for the standard drug clotrimazole, all test drugs and blank did not show antifungal activity against Aspergillus Niger.

Table 1: Ash value and Extractive value.

Drug powder	Total ash Value	Alcohol Insoluble Ash value	Water soluble ash value	Water soluble extractive value	Alcohol soluble extractive value	Chloroform soluble extractive value
Mimosa pudica shoot	6.45%	0.75%	0.45%	11.5%	10%	2.5%
Mimosa pudica root	7.15%	0.55%	0.35%	8.5%	7.5%	1.5%

Table 2: Percentage yield of MSE (Mimosa shoot extract)

Weight of drug	Extractive pattern	Solvent used	Weight Obtaining (gms)	Percentage yield
250 gms of Mimosa pudica shoot	Soxhlet apparatus	Petroleum ether	16gms	6.4%
		Chloroform	20gms	8%
		Methanol	13gms	5.2%
		Water	18gms	7.2%

Table 3: Percentage yield of MRE (Mimosa root extract).

Weight of drug	Extraction pattern	Solvent used	Weight Obtaining (gms)	Percentage yield
250 gms of mimosa pudica root	Soxhlet apparatus	Chloroform	18.5	7.4%
		Methanol	14	5.6%
		water	16.5	6.6%

Table 4: Chromatographic solvent system for MSE.

Solvent system	Ratio	Observation
Chloroform: Methanol	85:15	Separation7(pots)
Chloroform: Diethyl amine	90:10	Separation4(spots)
Toluene : Ethyl acetate : Diethyl amine	70:20:10	Separation2(spots)

Table 5: Chromatographic solvent system for MRE.

Solvent System	Ratio	Observation
Chloroform : methanol	85:15	Separation 5(spots)
Chloroform : diethyl amine	90:10	Separation 3(spots)
Toluene : ethyl acetate : diethyl amine	70:20:10	Separation 2(spots)

Table 6: TLC data analysis for MSE.

Detecting Reagent	Distance run by solvent	No. of Spots	Distance run by Solute	Rf Value	UV fluorescence at 254 nm
UV light	15cm	7	8	0.5333	Greenish black
			8.5	0.5666	Light orangish green
			9.3	0.6200	Light green
			10.4	0.6933	Light green
			12.8	0.8533	Light brown

			13.3	0.8866	Dark brownish green
			13.5	0.9000	Greenish brown

Table 7: TLC data analysis for MRE.

Detecting Reagent	Distance Run by solvent	No. of spots	Distance Run by solute	Rf Value	UV florescence at 254nm
UV light	14cm	5	7.5	0.5357	Light brown
			8	0.5751	Light green
			9.4	0.6714	Dark brown green
			10.5	0.7500	Brownish orange
			11.2	0.8000	yellow

Table 8: TLC data analysis for isolated compound MSME.

Detecting reagent	Distance run by solvent	No. of spots	Distance run by solute	Rf value	UV fluorescence at 254 nm
UV light	4.2cm	1	3.4	0.8095	Green color

Table 9: TLC data analysis for isolated compound MRME.

Detecting reagent	Distance run by solvent	No.of spots	Distance run by solute	Rf value	UV fluorescence at 254 nm
UV light	4.7cm	1	2.7	0.5744	Light brown green

Table 10: % yield of MSCE crystal.

Weight of drug	Solvent Used	Weight obtained (gms)	Percentage yield
1.5gm of crude MSCE	Chloroform	0.956mg	0.0637

Table 11: Phytochemical screening of MSME and MRME.

Phytochemical	MSME	MRME
Carbohydrate	+	+
glycoside	+	+
alkaloids	+	+
phytosterols	+	+
saponins	-	-
Fixed oil & fats	-	-
Tannins	-	-
Proteins and aminoacids	+	+
flavonoids	+	-
coumarins	+	-

(+) indicates positive test results

(-) indicates negative test results

Table 12: In-vitro Antibacterial activity of shoot extract of mimosa pudica.

Name of Micro organism	Name of compounds	Concentration (µg/disc)	Zone of Inhibition (mm)
Bacillus	MSME	10	20cm
	Gentamycin(std)	10	25mm
	MSCE	10	_
	MSWE	10	_
	Blank	-	_

Table 13: In-vitro Antibacterial activity of root extract of mimosa pudica.

Name of Micro organism	Name of compounds	Concentration (µg/disc)	Zone of Inhibition (mm)
Bacillus	MRME	10	15cm
	Gentamycin(std)	10	25mm
	MRCE	10	_
	MRWE	10	_
	Blank	-	_

Table 14: In-vitro Antifungal activity of shoot extract of mimosa pudica.

Micro organism	Name of Compounds	Concentration (µg/disc)	Zone of inhibition (mm)
Aspergillus Niger	MSME	10	_
	MSCE	10	_
	Clotrimazole	10	24mm
	MSWE	10	_
	Blank	-	_

Table 15: In-vitro Antifungal activity of root extract of mimosa pudica.

Micro organism	Name of Compounds	Concentration (µg/disc)	Zone of inhibition (mm)
Aspergillus Niger	MSME	10	_
	MSCE	10	_
	Clotrimazole	10	24mm
	MSWE	10	_
	Blank	-	_

Fig. 1: *Mimosa pudica* plant.

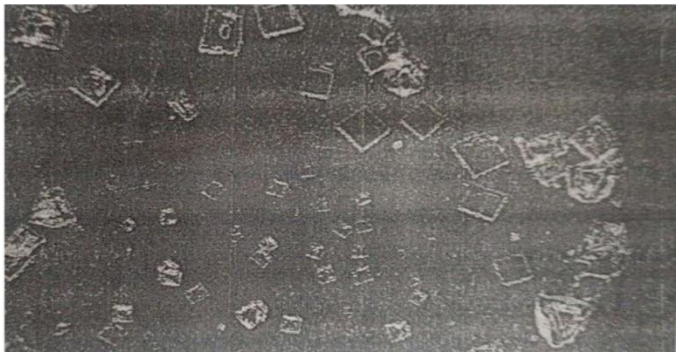

Fig. 2: Isolated crystal of MSCE.

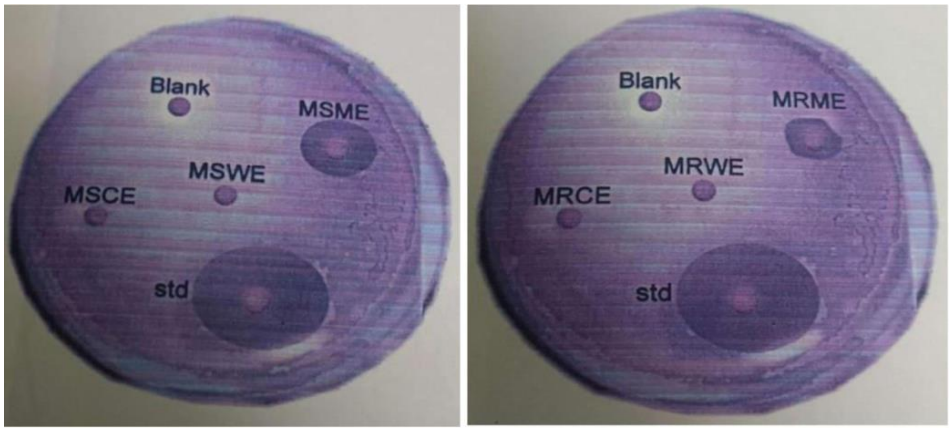

Fig 3: In-vitro Antibacterial activity of shoot and root extract of *mimosa pudica* against Bacillus.

4. CONCLUSION

The fresh shoot and root were collected and dried in shadow and subjected to hot continuous extraction by using Soxhlet apparatus with solvent petroleum ether, chloroform, methanol, and water. Pharmacognostical studies like histological studies (transverse section) and quantitative physical analysis of ash values, and extract value are performed. The chemical analysis shows the presence of carbohydrates, glycosides, alkaloids, and phytosterols are present. The TLC studies of MSME showed 7 spots the RF values ranging from 0.5333 to 0.9000 and MRME shows 5 spots with RF values ranging from 0.5377 to 0.8000 and the isolated single compound for MSME shows an RF value of 0.8055 and MRME show 0.5744. The antimicrobial activity of MSME, MRME, showed good antibacterial activity when compared with standard drug gentamycin and does not show antifungal activity.

5. Conflict of Interest

There are no conflicts of interest in this review study, the authors guarantee.

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