

INVESTIGATION OF PHYTOCHEMICALS AND IDENTIFICATION OF ACTIVE ANTIFUNGAL CONSTITUENT IN MIMOSA PUDICA LINN

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ABSTRACT

Mimosa pudica Linn., commonly known as the “sensitive plant,” is a well-known medicinal herb in traditional medicine, reputed for its antimicrobial, antioxidant, and wound-healing properties. This study aimed to investigate the phytochemical constituents of various extracts of *Mimosa pudica* Linn and to identify its active antifungal compounds through a systematic in vitro analysis. Methanolic and ethanolic extracts of the plant’s leaves were prepared and subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, phenols. The antifungal activity of methanol extract was evaluated against clinically relevant fungal strains such as , *Aspergillus flavus*, *Rhizopus species*. using the Disc diffusion method. The methanolic extract exhibited the most potent antifungal activity,

especially against *Aspergillus flavus*. Further fractionation and chromatographic analysis and spectroscopic analysis by using TLC, Column chromatography, FTIR, NMR, led to the isolation of bioactive compound quercetin being identified as major antifungal assays, confirming their role in the plant’s bioactivity. The findings support the ethnomedicinal use of *Mimosa pudica* suggest that its bioactive compounds could serve as potential leads in the development of novel antifungal agents.

KEYWORDS: *Mimosa pudica* L., Fungal Infection, Phytochemical screening, TLC, Column chromatography, FTIR, NMR, Antifungal activity.

INTRODUCTION

Mimosa pudica is well known for its rapid plant movement. In the evening the leaflets will fold together and the whole leaf droops downward. It then re-opens at sunrise.^[1] Nature has been a source of healing for thousands of years. For many years, many medicinal plants have been used in daily life around the world to treat diseases.^[2] *Mimosa pudica* was first formally described by Carl Linnaeus in *Species Plantarum* in 1753.^[3] *Mimosa pudica* is also known as chuimui^[4] or lajwanti in Hindi because of its unique property to droop or collapse when touched and opens up a few minutes later. Its other names are Betguen Sosa (Guam), Memege (Niue), Mechiuaiu (Palau), Limemeihr (Pohnpei), Ra Kau Pikikaa (Cook Islands). The Chinese name for this plant translates to "shyness grass".^[5] *Mimosa pudica* L. is a creeping annual or perennial herb. It has been identified as lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, and antidepressant properties. *M. pudica* is known to possess sedative, emetic, and tonic properties, and has been used traditionally in the treatment of various ailments including alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections. Phytochemical studies on *M. pudica* have revealed the presence of alkaloids, non-protein amino acid(mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids.^[6] In traditional medicinal systems, a variety of ethnomedicinal applications of *Mimosa pudica* Linn have been noticed.^[7]

Scientific Classification^[8]

Kingdom: Plantae

Division: Magnoliophyte

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Mimosoideae

Genus: *Mimosa*

Species: *M. pudica*

Geographical distribution

Mimosa pudica originates from South America and Central America. It is considered an invasive species in regions such as Tanzania, South Asia, Southeast Asia, and numerous Pacific Islands.^[9] In the Northern Territory, it is officially classified as a weed.^[10] Control

measures are advised in Queensland. Although it has been introduced to Nigeria, Seychelles, Mauritius, and East Asia, it is not viewed as invasive in those areas.^[11]

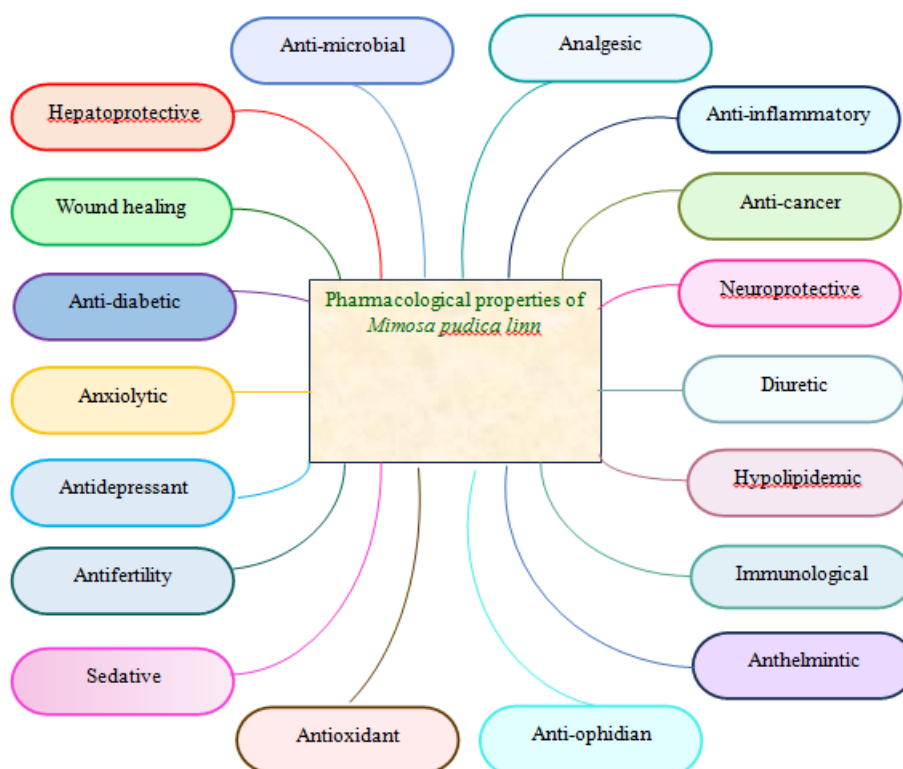


Figure No. 1: Pharmacological Properties of *Mimosa Pudica*.^[12]

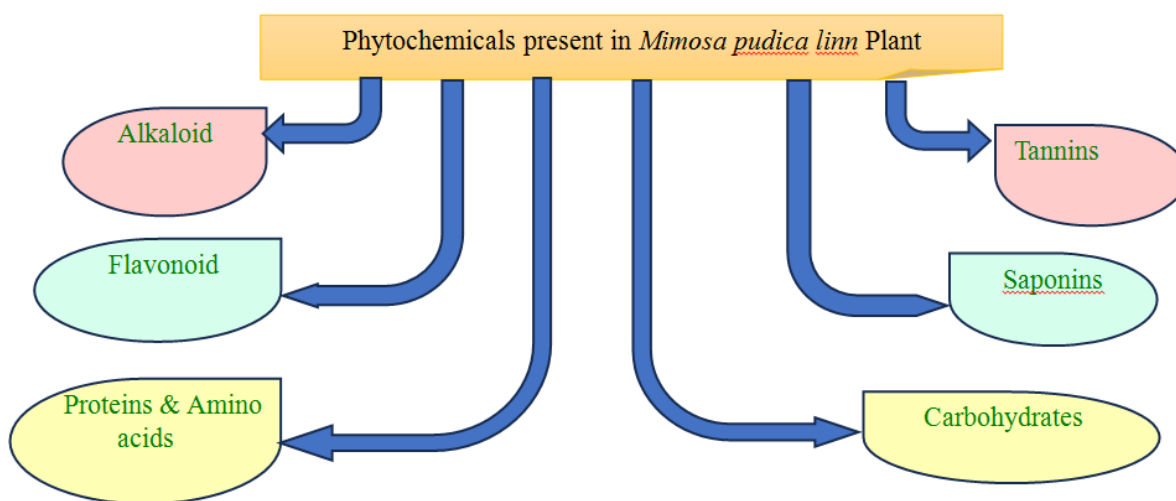


Figure No. 2: Phytochemicals present in *Mimosa pudica linn* Plant.

1.1. Skin

The largest exterior defense mechanism is the skin. Sensation, insulation, temperature regulation, vitamin D, metabolism & Vitamin B folate maintenance are among its other roles. The skin is the largest point of contact between the body & the environment with

thermoregulation, protection of the body against physical, chemical, microbiological harm & loss of water. The three are the main layers of skin are as

- a) Epidermis (Outermost layer)
- b) Dermis (Middle Layer)
- c) Hypodermis (Subcutaneous layer).^[14]

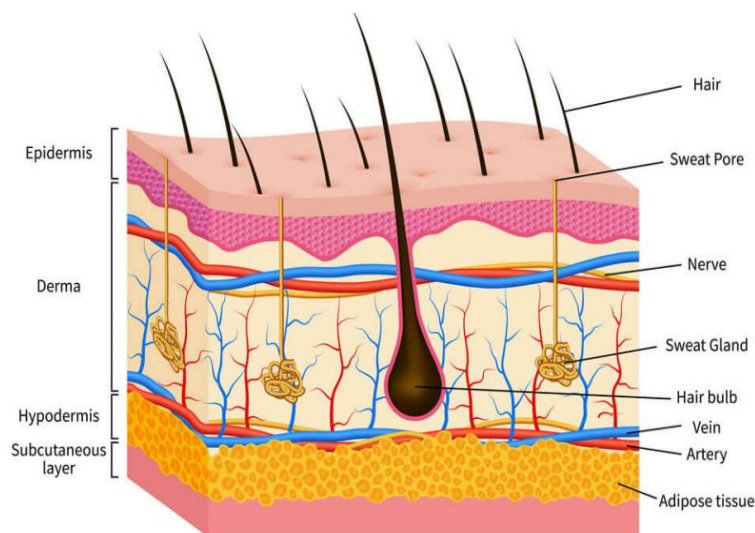


Figure No. 3: Anatomy of Skin layers.^[14]

The epidermis is the outer layer and is made up of keratinocytes, or skin cells, which form the “bricks” of our skin barrier. The role of the epidermis is to protect against environmental factors (such as UV rays and toxins), prevent drying, and maintain the immune system. The base of the epidermis is called the basal layer and contains cells that replicate the epidermis once a month. Interspersed between the epidermal keratinocytes are pigment cells called melanocytes, which give skin its unique color. These cells are activated when exposed to ultraviolet light from sunlight. The result of this activity is twofold - 1) melanocytes produce more melanosomes, producing envelopes containing brown melanin, and 2) it increases the number of melanosomes in neighboring people. The result is freckles or spots that can affect your appearance.^[15] The dermis is considered the core of the integumentary system. (dermis=skin), as particular from the epidermis (epi=upon or over) and hypodermis (hypo= underneath).^[16] The dermis, blood vessels and nourishes, smoothes and strengthens the epidermis. The dermis is divided into two parts: the upper layer papillary region and the deep reticular region. The upper papillary region is the thinner upper layer.

It consists of loose connective tissue in contact. Epidermal connective tissue has an extracellular matrix and fibroblasts. Fibroblasts also secrete fibronectin and Hyaluronic acid

is one of the two most important components that provide the skin with water retention. Matrix that plays a role in wound healing. The dermis also contains blood vessels, lymphatic vessels and epithelial cells, nerves and neurons.^[17] Hypodermis is also known as the subcutaneous layer or superficial fascia, this layer lies beneath the dermis and anchors the dermal part of the skin to the underlying fascia around the muscles or bones.^[18] The subcutaneous tissue is mainly composed of adipose tissue, which has the functions of fat storage, shock absorption and heat insulation. Richly vascularized and loose connective tissue of the areola. It is estimated that the easy and free movement of the underlying structure of the skin is due to the free arrangement of collagen and elastin fibers. The shock-absorbing function of the subcutaneous layer decreases with the process of fat storage aging.^[19]

1.2. Fungal infection

Fungal infections are of serious public health concern. The incidence of fungal infections in patients with other diseases including Covid-19 is associated with life-threatening mycoses and mortality. Fungal infections can include superficial, cutaneous, sub-cutaneous, mucosal and systemic infections with varying degree of severity. Organisms such as *Candida* spp. are part of human microbiota that can cause opportunistic infections in individuals and life-threatening infections (invasive candidiasis) in immuno-compromised patients such as HIV patients, cancer patients receiving chemotherapy, and patients receiving immuno-suppressive drugs.^[20] Fungal infections of the upper extremity are of four main types: cutaneous, subcutaneous, deep, and systemic. Cutaneous infections are caused by organisms capable of utilizing keratin.^[21] For medical purposes, fungal infections (mycoses) are classified as cutaneous (affecting the skin, fungal infections, hair and nails), subcutaneous and deep. Most fungi that cause skin diseases are

- a) Dermatophytes (*Trichophyton*, *Microsporum* and *Epidermophyton*)
which cause ringworm (dermatophytosis)
- b) *Candida* species which cause mucocutaneous candidiasis
- c) *Pityrosporum furfur*, also known as *Malassezia furfur*, which causes tinea versicolor^[22]



Figure No. 4: Fungal Infection.^[23]

Dermatophytes:-A class of keratinophilic fungi known as dermatophytes infects keratinised tissues, including the skin, hair, and nails. They are in charge of superficial fungal infections, also referred to as tinea infections or dermatophytoses. Traditionally, dermatophytes are divided into three main genera

- a) Trichophyton: Has an impact on nails, hair, and skin.
- b) Microsporum: Skin and hair are the main areas affected by microsporum
- c) Epidermophyton: Skin and Nails are affected by Epidermophyton.^[24]

Fungal spores can cause dermatophyte infections when they come into touch with the skin, particularly in warm, humid settings. The fungi break down keratin by secreting enzymes such as lipases, proteases, and keratinases, which makes it easier for them to infiltrate the skin's outermost layers. Typical clinical signs and symptoms include.

- a) Tinea corporis: Body ringworm b) Tinea pedis: Foot of the athlete



Figure No. 5: Body ringworm.^[25]



Figure No. 6: Foot of the athlete.^[26]

- c) Tinea cruris: Itching in the jock d) Tinea capitis: Ringworm of the scalp



Figure No. 7: Itching in the jock.^[27]



Figure No. 8: Ringworm of the scalp.^[28]

- d) Tinea unguium: Infections of the nails



Figure No. 9: Infection of the nails.^[29]

Red, Scaly, itchy sores that resemble rings are frequently the first signs to show.^[25] Plant-derived antifungal medicines are gaining popularity due to the increase in antifungal resistance. In vitro, some plant extracts have shown effectiveness against dermatophytes. For example: significant antifungal action against a variety of dermatophyte species is demonstrated by *Nigella sativa*. Curcumin, found in *Curcuma longa* (turmeric), has been demonstrated to prevent the growth of dermatophytes. Neem, or *Azadirachta indica*, exhibits broad-spectrum antifungal qualities. The antifungal properties of these plant extracts are facilitated by the presence of bioactive substances such as terpenoids, alkaloids, flavonoids, and saponins.^[30]

LITERATURE REVIEW

- 1) **Yusuf, et. al, 2025-** Phytochemical screening and neuro-pharmacological activity of *Mimosa pudica* flowers: integrating in vitro, in silico and in vivo approaches. *Phytopharmacol Phytochem.* 2025, In this, focused on methanolic flower extracts, identifying flavonoids, alkaloids, tannins, and saponins. The extracts demonstrated anxiolytic, antidepressant, and cognitive-enhancing effects in behavioral assays and docking studies pointed to MAO-A/B inhibition.^[31]
- 2) **Gandhi, et al. 2023-** Quantification of phytochemicals and metal ions as well as the determination of volatile compounds, antioxidant, antimicrobial and antacid activities of the *Mimosa pudica* L. leaf: exploration of neglected and under-utilized part. *Chem Biodivers.* 2023;20(10):e202301049. In this, *Mimosa pudica* L. (MP) is well-known plant in traditional medicinal system, especially in India. Unfortunately, leaves of MP are less explored. To determine the food and nutritional value of the neglected part of *Mimosa pudica* L. (MP), that is MP leaves, phytochemicals and metal ions of MP were quantified by newly developed HPLC and ICPOES-based methods. In summary, this study demonstrated the medicinal significance of MP leaves and the conversion of agro-waste or the under-utilized part of MP into pharmaceutical potent materials. Consequently, the present study highlighted that MP leaves alone have medicinal importance with good nutritional utility and possess large promise in the pharma industry along with improving bio-valorization and the environment.^[32]
- 3) **Shanti N, et.al 2023-** The Phytochemical potential of *Mimosa pudica* L plant under enhanced solar UV-B (280-320nm) radiation, *Applied ecology & Environmental sciences*,

11(4),2023,1300134, In this research, Because UV-B radiation increases the development of phenol, alkaloids, saponin, and other secondary bioactive chemicals, it can enhance the quality of medicinal plants like *Mimosa pudica* L. TLC qualitative analysis of these chemicals showed that they were present in high amounts, with plants exposed to UVB radiation producing more of them. Increased FRAP activity and antioxidant potential are correlated with the formation of these phytochemical substances. Investigating the possible uses of these enhanced bioactive compounds in pharmaceutical research and development is the long-term objective.^[33]

- 4) **Ganiga C. Shivakumar, *et.al.* 2023** - Analytical Study on Current Trends in the Clinico-Mycological Profile among Patients with Superficial Mycoses, Journal of Clinical Medicine, 2023,12,3051. In this article their study on the current trends in the clinico-mycological profile among patients with superficial mycoses in Eastern Odisha, a hot and humid part of the country, revealed that the age range of 11–20 years had the highest prevalence of SM out of the 250 clinically confirmed cases of the condition included. Furthermore, they found that dermatophytes were the most prevalent superficial fungal species, accounting for 73% of the total cases. So future research can focus on developing more sensitive and specific diagnostic methods for superficial mycoses to improve patient outcomes.^[34]
- 5) **Mukherjee & Ramesh, *et.al.* 2022**- studied root extracts, revealing compounds including toxic alkaloids like mimosine, orientin, isoorientin, D-pinitol, tannins, C-glycosides, steroids, terpenoids, fatty acids, coumarin. They confirmed significant in vitro anthelmintic activity.^[35]
- 6) **Komal Rizwan, *et.al.* 2022** - Phytochemistry and Diverse Pharmacology of Genus *Mimosa*: A Review, Biomolecules, 2022,12,83. This review summarized the isolated phytochemical and pharmacological characteristics of the *Mimosa* genus. Out of 400 species only 25 have been chemically studied, while compounds belonging to different chemical classes have been isolated in the *Mimosa* species, such as alkaloids, chalcones, flavonoids, indoles, terpenes, terpenoids, saponins, steroids, amino acids, glycosides, flavanols, phenols, lignoids, polysaccharides, lignin's and fatty esters. So, there is a need to provide detailed mechanistic studies on the pharmacology to provide a good

understanding of the application of the *Mimosa* species as a source of potential medicines.^[36]

- 7) **Suresh Mickymaray, *et.al.* 2020** - Anti-Fungal Efficacy and Mechanisms of Flavonoids, Antibiotics, 2020,9,45. In this Article includes, Various flavonoids have been extracted and investigated in association with their anti-fungal activities and can be promising, efficient, and cost-effective agents for the inhibition of fungal infections. They often inhibit fungal growth in various underlying mechanisms by enhancing the disruption of the plasma membrane and mitochondrial dysfunction; and inhibiting cell wall formation, cell division, protein synthesis, and the efflux-mediated pumping system. These flavonoids are capable and efficient in synergetic combination therapy with conventional drugs, which can be more appropriate and supportive for finding novel drug therapies against fungal pathogens.^[37]
- 8) **Vijayalakshmi K, *et.al.* 2018** - Antifungal Activity of *M. pudica* L. Against Selected Human Pathogens, International Journal of Advanced Scientific Research and Management, Volume 3 Issue 10, Oct 2018, in this study, the antifungal activity of acetone extract, Aqueous extract, benzene extract, ether extract and ethanol extract Leaves and roots of *M. pudica* for selected fungal species *Aspergillus terreus*, yellow yeast, black yeast and *fusarium Sorani*. Antifungal properties of medicinal plants *M. pudica* may be due to the presence of bioactive substances combine. More research is needed to know bioactive compounds responsible for Antifungal activity.^[38]
- 9) **Alamgir, *et.al* 2017-** Therapeutic use of medicinal plants and their extracts: Pharmacognosy, vol 1, In this research, Flavonoids and tannins, in particular, have been noted for their ability to disrupt microbial cell walls and inhibit fungal enzymes system.^[39]
- 10) **Prosanta Pal, *et.al* 2015** - Phytochemical Analysis of The Whole Plant of *Mimosa pudica* (Linn.), UJPSR / 1 (1), 2015, 1-9, In this Research The antiasthmatic, aphrodisiac, analgesic, and antidepressant, sedative, emetic, alopecia, diarrhea, dysentery, insomnia, tumor, and various urogenital infections can be attributed to their high alkaloids, proteins, amino acids, tannins, phenolics, flavonoids, steroids and saponins. Among these compounds alkaloids were separated and identified in the mother extract and methanolic fraction of 50% ethanolic extract of *Mimosa pudica* (Linn.) as alkaloids were found to

possess different pharmacological activity. Hence in future it will be aimed to isolate the alkaloidal compounds from methanolic fraction and to screen their pharmacological activities in in vitro and in vivo models.^[40]

Plant Profile

Taxonomy^[41]

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae
- Genus: *Mimosa*
- Species: *Mimosa pudica*

Common Names^[42]

- English: Sensitive Plant, Touch-Me-Not, Shameplant
- Hindi: Chui-muli
- Sanskrit: Lajjalu
- Tamil: Thottavadi
- Malayalam: Thottavadi

Botanical Description

- Habit: Herbaceous, creeping or trailing perennial
- Height: 30–100 cm^[43]
- Leaves: Alternate, bipinnately compound with 10–26 leaflets per pinna; sensitive to touch, folding inward upon stimulation
- Flowers: Small, globose, pink to purple inflorescences, 8–10 mm in diameter^[44]
- Fruits: Flat, clustered pods (legumes), containing 2–5 seeds, with small prickles
- Roots: Taproot system

Distribution and Habitat^[44]

Native to: Central and South American tropical and subtropical regions Naturalized in: Tropical and subtropical areas across the world, such as Australia, South Africa, West Africa, Micronesia, East Asia, and South and Southeast Asia Habitat: Found in disturbed places, roadsides, wastelands, and open fields; favors full sun and moist, well-drained soils.

Phytochemistry

- Alkaloids: Mimosine
- Flavonoids: Quercetin, Kaempferol
- Tannins: Tannic acid
- Glycosides: Mimoside
- Terpenoids: Lupeol
- Steroids: β -sitosterol

Medicinal Uses**Traditional Uses^[46]**

- Ayurveda: Used for treating biliousness, leprosy, skin diseases, wounds, diarrhea, dysentery, vaginal and uterine ailments, inflammations, and asthma.
- Folk Medicine: Decoction of the root is used for treating various ailments; paste of leaves applied to relieve glandular swellings.
- Unani: Used for similar purposes as in Ayurveda.
- Tibetan Medicine: Used for treating various ailments.

Pharmacological Properties^[47]

- Antioxidant: Exhibits free radical scavenging activity
- Antibacterial: Effective against various bacterial strains
- Antifungal: Inhibits fungal growth
- Anticancer: Contains compounds with potential anticancer activity

MATERIALS AND METHODS**6.1 Plant collection & Preparation**

- 1) Plant material- *Mimosa pudica* (Linn.) This plant was found in tropical and subtropical regions of India. It occurs in barren lands, especially where there is humid and hot weather. Leaves part of *Mimosa pudica* linn was used.



Figure No. 10: *Mimosa pudica linn* plant.

- 2) Plant collection- The Plant material is collected from Sindhudurg district. It was identified & confirmed by the Department of Botany, Anandibai Raorane Arts, Commerce & Science College, Vaibhavwadi, Sindhudurg.
- 3) Drying & Grinding- The leaves material *Mimosa pudica linn* was dried in shade & the dried material coarsely powdered by means of mechanical grinder. The resulting powdered material used for further studies.



Figure No. 11: Drying of *Mimosa* plant leaves.



Figure No. 12: Powder of *Mimosa* plant leaves.

6.2 Extraction Methods

- 1) Method 1 (Maceration) - 50 gram of leaf and root powder of *M. pudica* were soaked in 500 ml of ethanol and then kept in orbital shaker for 48 h at room temperature. After 48 h, the mixture was filtered through a clean muslin cloth. Then the filtrate again filtered by using a Whatman No. 1 filter paper and the extracts were concentrated and dried in a rotary evaporator at 37 °C till a sticky mass was obtained. After evaporation of solvents, the dried extracts were stored at 4 °C until further use.^[48]



Figure No. 13: Maceration of *Mimosa* leaves powder.

2) Method 2- The leaf powder (about 50 g) was defatted with petroleum ether and then loaded into a Soxhlet extractor and extracted with 500 ml methanol at room temperature. After completion of extraction for 72 h, the solvent was removed by distillation and the concentrated extract was dried under reduced pressure at 40 Â°C in a field evaporator. A thick semi-solid brown paste was obtained and stored in a desiccator at room temperature.^[49]



Figure No. 14: Extraction of *Mimosa* leaves powder.

6.3 Phytochemical Screening^[50,51]- The Phytochemical screening of the extract was conducted by qualitative tests.

Table No. 2: Phytochemical screening.

Sr. no.	Phytoconstituents Name	Test	Observation	Inference
1.	Alkaloid	Meyer's test - 2 ml of extract was taken in a test tube & 0.2 ml dilute HCL was added, add 1ml of Meyer's reagent.	Yellowish color appears.	Alkaloid is present

		Dragendorff's/ Kraut's test- Few mL filtrate + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate	Alkaloid is present
		Wagner's test- Few mL filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate	Alkaloid is present
2.	Glycoside	A small amount of alcoholic extract was taken in 1ml of water in a test tube & a few drops of Aq. NaOH were added.	Yellow color appears	Glycoside is present
		Keller-Killani test- 1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H ₂ SO ₄ (along the side of test tube)	A blue coloured solution (in acetic acid layer)	Glycoside is present
3.	Flavonoid	1-5 drops of conc. HCL add to little amount of methanolic extract of plant material.	Immediately develop Red color	Flavonoid is present.
		Alkaline reagent test 1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid	Flavonoid is present
		Alkaline reagent test- Plant extract + 10% ammonium hydroxide solution.	. A yellow fluorescence	Flavonoid is present
		Lead acetate test 1mL plant extract + few drops of 10% lead acetate solution	A yellow precipitate	Flavonoid is present.
		Ferric chloride test Extract aqueous solution + few drops 10% ferric chloride solution.	A green precipitate	Flavonoid is present.
		Pew's test Few mL aqueous extract solution + 0.1gm metallic zinc + 8mL conc. H ₂ SO ₄	A red colour	Flavonoid is present
4.	Phenol	Ferric chloride test- Add a small amount of methanolic extract with water in a test tube & add 1-2 drops of Iron III Chloride (FeCl ₃).	Blue/ Green/ Red/ purple color appears.	Phenol is present
		Iodine test - 1mL extract + few drops of dil. Iodine sol.	A transient red colour	Phenol is present

6.4 Advanced Characterization- The detection of flavonoid was detected by Chromatography & Spectroscopy.

6.4.1 Chromatography Techniques

6.4.1.1 Thin-Layer Chromatography

Pipette 10 microliters of the sample solution and reference solution, respectively, onto the silica gel slate that has CMC Na adhesive on it in accordance with the TLC specified in Chinese Pharmacopoeia version 2010 appendix VIB. As a developing solvent, use 5:4:1, Toluene: Ethyl acetate : Formic acid. Place the slate in the saturated state of the developing chamber. Once it has developed, remove it and let it air dry. The sample solution's chromatograph shows the same color speckle at the same location as the standard solution.^[52]

6.4.1.2 Column Chromatography

The partial purification of methanol extract was carried out by the sequential purification through column chromatography. Activated silica gel (200 g, 60-200 mesh) was used as a stationary phase and Butane in sequence were used as a mobile phase. The crude methanol residue immiscible in butane and was triturated with silica and further column chromatography was carried out. First benzene fraction was collected followed by methanol fractions. To confirm the separation, thin layer chromatography was conducted using a sample with the mobile phase. The analysis of flavonoids was carried out on TLC plates coated with silica gel, utilizing 10 µl of the sample. The mobile phase for TLC was toluene, ethyl acetate, and formic acid (7:5:1), and the fractions were subsequently screened for flavonoids.^[53,54]

6.4.2 Spectroscopy Techniques

1. FTIR Analysis- Certain frequencies of infrared light will be absorbed as they go through an organic compound sample, while other frequencies will pass through the sample unabsorbed. The vibrational changes that take place inside a molecule when it is exposed to infrared radiation are associated with infrared absorption. Thus, infrared spectroscopy can be essentially described as vibrational spectroscopy. The vibrational frequencies of the C–C, C=C, C–O, C=O, O–H, and N–H bonds vary. The distinctive frequency absorption band in the infrared spectrum can be seen if an organic molecule contains these kinds of links. A high-resolution analytical technique for identifying chemical constituents and elucidating structural structures is Fourier Transform Infrared

Spectroscopy (FTIR). FTIR offers a rapid and non-destructive way to examine the fingerprints of powdered herbs or extracts.^[55,56]

2. NMR Spectroscopy

¹H NMR spectra were acquired on a Bruker DSX-300 spectrometer, using the standard pulse program '1c1pnf2', which is based on the one dimension version of the NOESY sequence and allows double pre-saturation, to suppress the water peaks. 32k data points were recorded over a sweep width of 9191 Hz, with 512 scans. An exponential line broadening of 1 Hz was imposed on the accumulated data before Fourier transformation. The ¹³C NMR experiments were obtained at 400.23 MHz on a Bruker Biospin Ultrashield plus AV-400 MHz instrument.^[57]

6.5 Antifungal Activity testing

6.5.1 Fungal Strains- *Aspergillus flavus* and *Rhizopus* species were used to testing the antifungal activity of leaves extract of *Mimosa pudica* linn.

RESULTS AND DISCUSSION

7.1 Preliminary Phytochemical test- Preliminary qualitative phytochemical analysis of *Mimosa pudica* linn extracts(Methanol & Ethanol) revealed the presence of various secondary metabolites (Table 3). The methanol & ethanol extracts showed strong presence of flavonoid, alkaloid & phenol.

Table No. 3: Phytochemical constituents detected in *Mimosa pudica* leaf extracts.

Sr. No.	Test Name	Methanol	Ethanol	Phytochemical
1.	Meyer's test	+	+	Alkaloid
2.	Dragendroff's test	+	+	Alkaloid
3.	HCL test	-	-	-
4.	Alkaline reagent test	+	+	Flavonoid
5.	Shinoda test	++	++	Flavonoid
6.	Lead acetate test	+	-	Flavonoid
7.	Ferric Chloride test	+	+	Flavonoid
8.	Pew's test	+	-	Flavonoid
9.	Ferric chloride test	+	+	Phenol
10.	Iodine test	+	+	Phenol

Note: ++= Strong presence, + = Presence, - = Absence



Figure No. 15: Shinoda test for Flavonoid.

7.2 Isolation & Characterization

The successful isolation and structural characterization of quercetin from *Mimosa pudica* linn validate the plant's potential as a source of bioactive flavonoids. Quercetin is well-documented compound known for a wide range of biological activities.

The combination of TLC, Column chromatography, FTIR, NMR Provided strong confirmation of the identity of quercetin in the methanolic extract of *Mimosa pudica*.

The efficiency of methanol in extracting flavonoid-rich fractions supports earlier findings that methanol is superior to ethanol solvents for polyphenol extraction.

Moreover, the high yield & purity of the isolated quercetin highlight the feasibility of using *Mimosa pudica* as a sustainable source for natural antifungal agents. Its antifungal activity, discussed in subsequent sections, underscores its potential therapeutic value.

7.2.1 Isolation

7.2.1.1 TLC

Solvent system (Mobile phase) - Toluene: Ethyl acetate: Formic acid (5:4:1)

Stationary phase – Silica gel G.

To compare the yellow spot in both lane A and lane B, we'll focus only on those yellow pigments that appear visually similar. The yellow spot in lane A (upper yellow band) and the yellow spot in lane B (only spot) appear similar in color and height. This shows that they have the same compound. i.e may be. quercetin.



Figure No. 16: TLC of *Mimosa* leaf methanol extract.

Rf Value = Distance traveled by compound (spot) / Distance traveled by solvent front

Distance traveled by compound from baseline = 1.6

Distance traveled by solvent front from baseline = 2.7

Rf value = $1.6/2.7$

Rf value = 0.59

The Rf value indicate that the yellow spot was may be quercetin compared with Standard sample spot on TLC plate.

7.2.1.2 Column chromatography

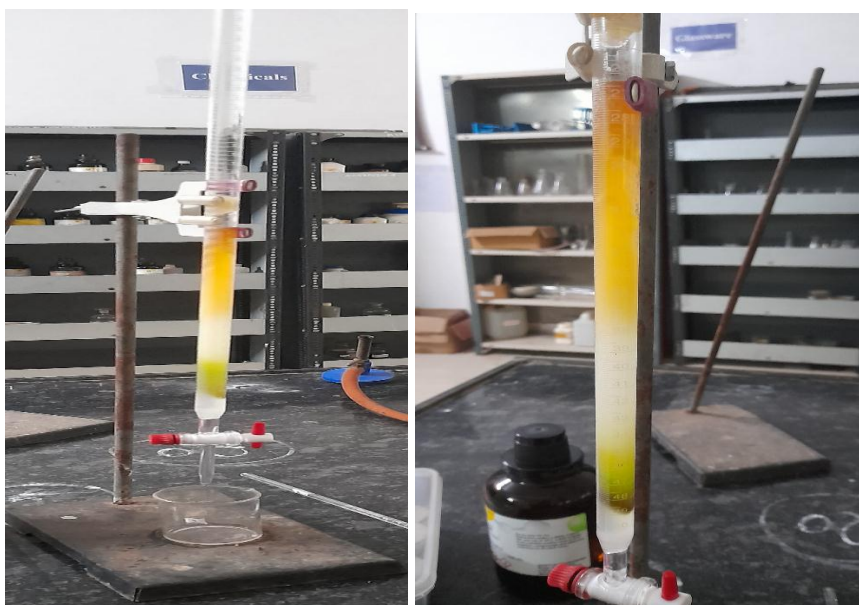


Figure No. 17: Column Chromatography of Methanol extract.

The partial purification of methanol extract was carried out by the sequential purification through column chromatography. Activated silica gel (200 g, 60-200 mesh) was used as a stationary phase and Benzene in sequence were used as a mobile phase. The crude methanol residue immiscible in butane and was triturated with silica and further column chromatography was carried out. First benzene fraction was collected followed by methanol fractions. The Column chromatography of methanolic extract of *Mimosa pudica* leaves was performed by using Silica gel as the stationary phase & Benzene as the mobile phase. The column showed clear separation of phytoconstituents, observed as distinct colored bands.

- Top band- Orange to red
- Middle band- Bright yellow
- Bottom band- Pale yellow to off-white

The presence of a distinct bright yellow band suggests the likely presence of flavonoid in Methanol extract of *Mimosa pudica* leaves.

7.2.2 Characterization

7.2.2.1 FTIR

The FT-IR spectrum of isolated compound was shown in figure no. 18 and their corresponding characteristic peak positions were listed in table no. 4. C-H stretching were observed at 2945 cm^{-1} . The broad absorption peak at around 3921 cm^{-1} was assigned to the OH stretching vibration of Phenolic compound. C=O aryl ketonic stretching vibration are observed at 1691 cm^{-1} . OH bending vibrations of phenols were observed at 1391 cm^{-1} . The lower frequency 865 cm^{-1} were assigned to the C-H bending vibrations of aromatic ring. C-X bending were observed at 431 cm^{-1} .

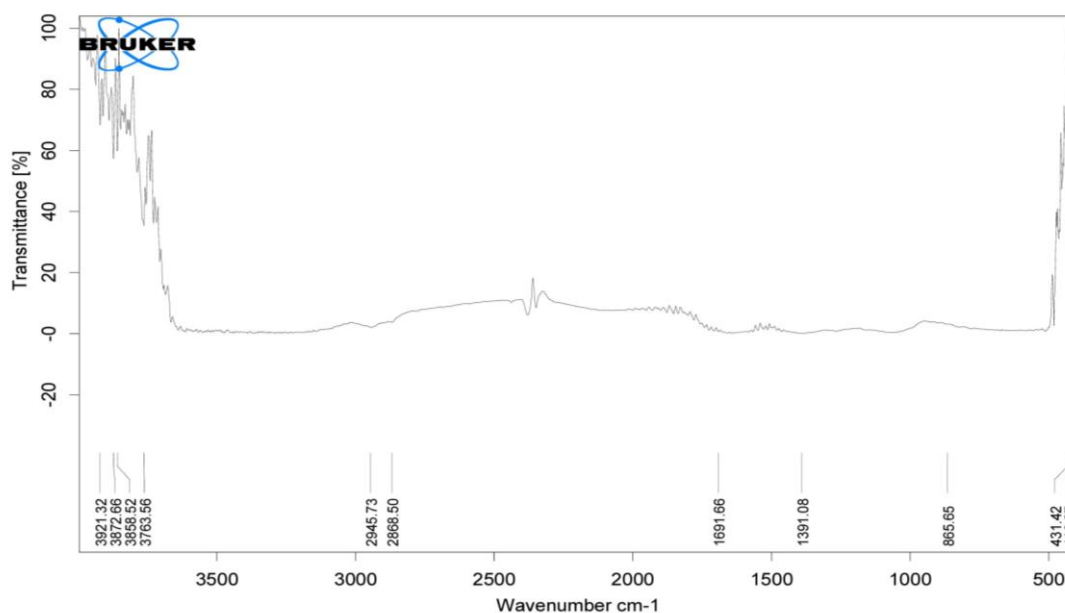


Figure No. 18: FTIR graph of Isolated compound.

Table No. 4: FTIR Analysis of Isolated compound.

Wavenumber (cm ⁻¹)	Functional group/Bond	Type of vibration	Probable functional groups
3921,3908,3885,3876	O – H (Phenolic or alcohol)	Stretching (broad region)	Phenolic compounds, flavonoids
2945, 2888	C – H (alkane, methyl/methylene)	Stretching	Aliphatic chain
1691	C=O (Carbonyl group)	Stretching	Aldehydes, ketones, carboxylic acids
1391	O – H bending / C – O – H	Bending (phenolic)	Phenolic – OH or C – H bending vibration
865	C – H (aromatic)	Out – of – plane bending	Aromatic ring
431, 418	C – X (Cl, Br or ring deformation)	Bending	Aromatic ring deformation or presence of halogens

7.2.2.2 NMR Spectra analysis

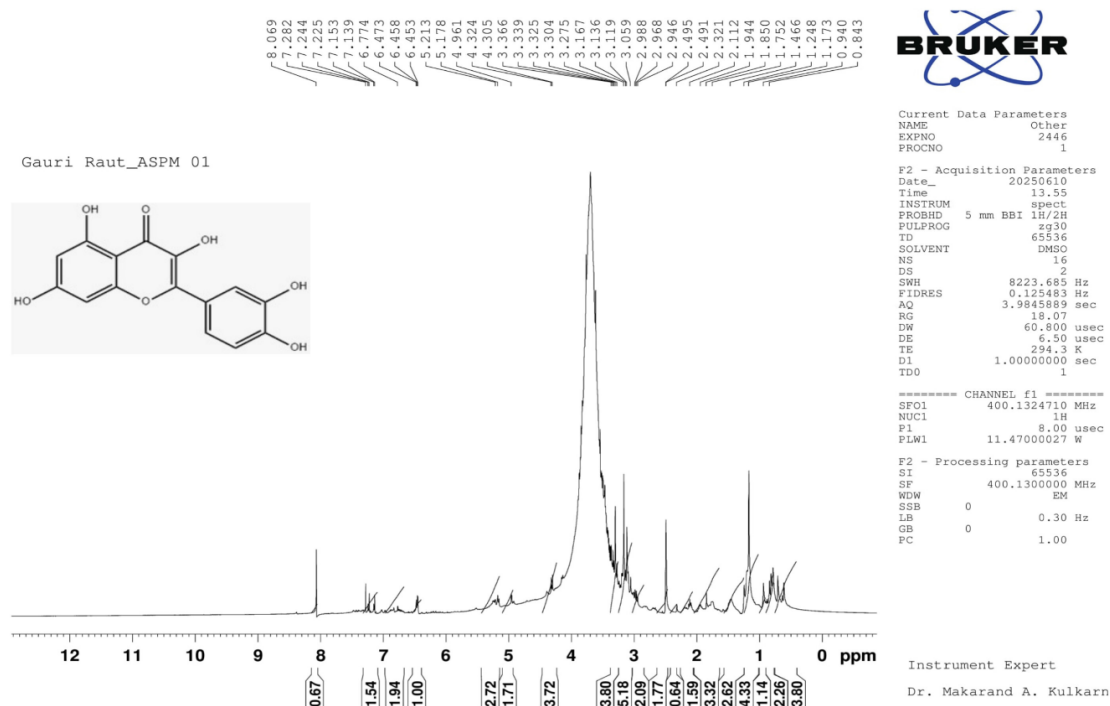


Figure No. 19: ^1H NMR Spectra of Isolated component.

NMR studies were carried out to confirm the positions of proton and carbon binding sites. The isolated compound displayed a better resolved ^1H -NMR spectrum in figure no. 19 and in table no. 5. The ^1H -NMR spectrum of the isolated compound showed 7.153 δ ppm observed triplet proton at C₂, C₅, C₆, and the broad single peak at 3.167 δ ppm were showed aromatic hydroxy group at C₂, 5.213 δ ppm were showed doublet proton at C₅, C₇. So this all over analysis may showed presence of Quercetin.

Table No. 5: ^1H NMR Analysis of Isolated component.

^1H NMR (DMSO δ ppm)	7.153 (m Ar - 3H = C ₂ , C ₅ , C ₆), 3.167 (s Ar - OH = C ₃), 5.213 (d Ar - 2H = C ₆ , C ₈)
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7.3 Antifungal Assay

The results validate the antifungal potential of quercetin isolated from *Mimosa pudica*. The increase in zone of inhibition from crude extract to purified compound clearly indicates that quercetin is a major active antifungal constituent. The mechanisms by which quercetin exerts antifungal effects may include

- I. Disruption of fungal cell wall or membrane integrity
- II. Inhibition of ergosterol biosynthesis

III. Generation of reactive oxygen species (ROS) causing oxidative stress in fungal cells.

These modes of action have been reported in literature for quercetin and flavonoid compounds.

Antifungal Assay report-

Name of test- Disc Diffusion Assay (Quantity per disc-10 μ l)

Inoculum used- 1×10^6 CFU/ml

Incubation Temperature- 30 °C

Incubation time- 24 hrs

Growth Media- Sterile Potato Dextrose Agar at pH 5.1

Test bacterial culture- *Aspergillus flavus* and *Rhizopus sp.*

RESULT

Table No. 7: Antifungal Assay report.

Sample ID	Inhibition zone diameter (mm) against pathogen	
	<i>Aspergillus flavus</i>	<i>Rhizopus sp</i>
Sample	15	---
Methanol control	09	07

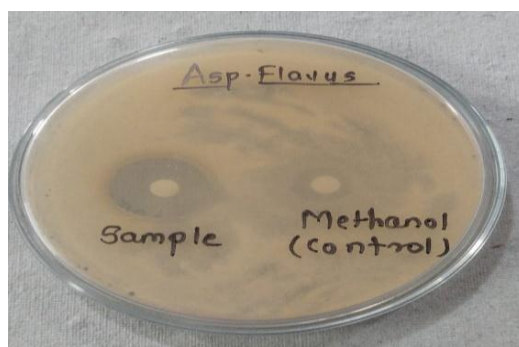


Figure No. 20: Antifungal assay report with *Aspergillus flavus*.

In fig. No.22, the sample and methanol used as a control was tested against *Aspergillus flavus* Fungal species by Disc diffusion method. The sample showed a clear inhibition zone of 15mm against *Aspergillus flavus*, which is significantly larger than the methanol control zone of inhibition of 9mm. This confirms that the observed antifungal effect is due to the bioactive constituent in the *Mimosa pudica* extract, not the solvent.



Figure No. 21: Antifungal assay report with *Rhizopus* species.

For *Rhizopus* species, there was no inhibition observed by the sample, while methanol control showed a slight inhibition of 7mm showed in Fig. No. 23. This small zone may be due to mild solvent effect but since the sample showed no additional inhibition, it indicates no significant antifungal activity against *Rhizopus* species at the tested concentration.

The result strongly suggests that *Mimosa pudica* contains active phytoconstituent i.e. Quercetin flavonoid that was selectively active against *Aspergillus flavus*. The extracts lack of activity against *Rhizopus* suggests a spectrum-specific antifungal effect.

CONCLUSION

The present investigation into the phytochemical constituent of *Mimosa pudica* linn. Has revealed the presence of various bioactive compounds, including alkaloid, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its therapeutic potential.

Among these, flavonoids were found to exhibit significant antifungal activity, particularly against common fungal pathogens such as and *Aspergillus flavus*. The antifungal efficacy demonstrated by the crude extracts and partially purified fractions suggests that *Mimosa pudica* is a promising source of natural antifungal agents.

This study successfully demonstrated that *Mimosa pudica* Linn contains significant amounts of quercetin, a bioactive flavonoid with potent antifungal activity. Phytochemical screening confirmed the presence of various secondary metabolites, particularly flavonoids. Through chromatographic and spectroscopic methods, quercetin was isolated and identified as a major constituent of the methanolic extract.

The isolated quercetin exhibited substantial antifungal activity against *Aspergillus flavus* surpassing the activity of the crude extract. Further isolation and structural characterization of

the active constituents, along with in-depth pharmacological studies, are essential to validate their potential for the development of plant-based antifungal therapies.

These findings support the ethnopharmacological use of *Mimosa pudica* in traditional medicine and suggest that quercetin may serve as a promising lead compound for the development of natural antifungal agents. This study supports the traditional use of *Mimosa pudica* in treating fungal infections and highlights its relevance in the search for novel phytotherapeutic agents. Further in Vivo studies and toxicity evaluations are recommended to assess its therapeutic viability and safety profile.

BIBLIOGRAPHY

1. Raven, Peter H. Evert, Ray F, Eichhorn, Susan E. Physiology of Seed Plants: 29. Plant Nutrition and Soils. Biology of Plants (7th Ed.), New York: W. H. Freeman and Company, p. 639.
2. Nair R, Kalapriya T, Sumitra C. Antibacterial activity of some selected Indian Medicinal Flora Turk. J. Bot, 2005; 29: p.41-47.
3. Saraswat R, Pokharkar R. GC-MS Studies of *Mimosa pudica*. International Journal of PharmTech Research 12, 4(1): p.93-98.
4. Chauhan, Bhagirath S. Johnson, Davi E. Germination, emergence and dormancy of *Mimosa pudica*. Weed Biology and Management, 2009; 9(1): p.38-45.
5. *Mimosa pudica*. Usambara Invasive Plants. Tropical Biology Association. Retrieved, 2008; p.3-25.
6. Genest S, Kerr C, Shah A, Rahman MM, Saif-E-Naser GM, Nigam P, et al. Comparative bioactivity of two *Mimosa* species. Lat Am Carib Bull Med Aromat Plants, 2008; 7: p.38-43.
7. Kumar, Vijay, Phytochemical, Pharmacological Activities and Ayurvedic Significances of Magical Plant *Mimosa pudica* Linn, Mini-review in organic Chemistry, 2021; 18(3): p.296-312(17).
8. George J., Joseph B., Mohan J., Pharmacology and Traditional Uses of *Mimosa pudica*, International Journal of Pharmaceutical Sciences and Drug Research, 2013; 5(2): p.41-44.
9. Adurosakin O., Chinedu S., Dike E., Iweala E., Otake J., Owanta J., Uche M., Ugbogu E., Ugbogu O., Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicological effects of *Mimosa pudica* - A review, Pharmacological Research - Modern Chinese Medicine, 2023; 7: p.100241.

10. *Mimosa pudica*. Usambara Invasive Plants. Tropical Biology Association. Retrieved, 2008; p.3-25.
11. Declared Weeds in the NT - Natural Resources, Environment and The Arts. Archived from the original on 2008-02-26. Retrieved, 2008; p.3-25.
12. Invasive plants and animals. Biosecurity Queensland. Archived the original on 2009-04-19. Retrieved, 2008; p.3-25.
13. Yadav RNS. Agarwala M., Phytochemical analysis of some medicinal plants. *J Phyto*, 2011; 3(12): p.10-14.
14. Tais G, Ulrich S, Lihong J, mingyann G, et al. Penetration of quantum dot particles through human skin, 2010; 6: p.586-595.
15. Zahra Lotfollahi, The anatomy, physiology and function of all skin layers and the impact of ageing on the skin, School of Clinical Sciences, Monash University, Clayton, Australia, Available from - <https://doi.org/10.33235/wpr.32.1.6-10>
16. Biga LM, et al, *Anatomy & Physiology*. 1st ed. OpenStax; 2019.
17. Sussman C, Bates-Jensen B. Skin and soft tissue anatomy and wound healing physiology. In: Sussman C, Barbara M, Bates- Jensen B, editors. *Wound care: A collaborative practice manual for physical therapists and nurses*. Philadelphia, PA: Lippincott Williams & Wilkins, 2012; p. 17–52.
18. Monteiro-Riviere NA. *Toxicology of the skin*. Boca Raton: Informa Healthcare; 2010.
19. Bonham CA, Kuehlmann B, Gurtner GC. Impaired neovascularization in aging. *Adv Wound Care (New Rochelle)*, 2020; 9(3): p.111–126.
20. G. Kiran Kumar Reddy, Alwar Ramanujam Padmavathi, Y.V. Nancharaiah, Fungal infections: Pathogenesis, antifungals and alternate treatment approaches, *Current Research in Microbial Sciences*, 2022; vol 3.
21. Thomas F. Hitchcock MD, Peter C. Amadio MD, Fungal Infections, *Hand Clinics*, November 1989; 5(4): p.599-611.
22. Bhandarkar SD, Rege NN, Satoskar RS, *Pharmacotherapy of common skin disorders and skin Protectives, Pharmacology and Pharmacotherapeutics*, Revised 20th Edition, p.1000-1002.
23. Available, from-
https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.icliniq.com%2Farticles%2Fskincare%2Fsuperficialfungalinfections&psig=AOvVaw1EbvF1BACly_sqNr2XDRiv&ust=1735380005669000&source=images&cd=vfe&opi=89978449&ved=0CBUQjhxqFwoTCOilx7nYx4oDFQAAAAAdAAAAABAE

24. Moskaluk A. E, Vandewoude S, Current topics in Dermatophyte classification and clinical diagnosis, *Pathogens*, 2022; 11(9): p.957.
25. Available from - <https://dermnetnz.org/imagetdetail/9964-tinea-corporis>
26. Available from - https://www.medicinenet.com/image-collection/ringworm_tinea_pedis_picture/picture.htm
27. Available from- <https://images.app.goo.gl/xsMdrzbhd3a3C5oWA>
28. Available from- https://en.wikipedia.org/wiki/Tinea_capitis
29. Available from - <https://images.search.yahoo.com/search/images?p=funhgal+infdection+of+tinia+unguim+images&fr=mcafee&type=E210US885G0&imgurl=https%3A%2F%2Fi.ytimg.com%2Fvi%2FTuWsT9pqnt8%2Fmaxresdefault.jpg#id=3&iurl=https%3A%2F%2Fi.ytimg.com%2Fvi%2FTuWsT9pqnt8%2Fmaxresdefault.jpg&action=click>
30. Bains A, Chawla P, Dhull SB, Kaur N, Kaushik R, Melinda F, A review on antifungal efficiency of plant extracts entrenched polysaccharide-based nanohydrogels, *Nutrients.*, 2021; 13(6): p.2055.
31. Yusuf ATM, et al. Phytochemical screening and neuro-pharmacological activity of *Mimosa pudica* flowers: integrating in vitro, in silico and in vivo approaches. *Phytopharmacol Phytochem.* 2025.
32. Gandhi Y, Kumar V, Singh G, Prasad SB, Mishra SK, Soni H, et al. Quantification of phytochemicals and metal ions as well as the determination of volatile compounds, antioxidant, antimicrobial and antacid activities of the *Mimosa pudica* L. leaf: exploration of neglected and under-utilized part. *Chem Biodivers*, 2023; 20(10): e202301049.
33. Kumar V, Mani PJ, Murugesan S, Shanthi N, The phytochemical potential of *Mimosa pudica* L plants under enhanced solar UV-B (280-320nm) radiation, *Applied Ecology and Environmental Sciences*, 2023; 11(4): p.130-134.
34. Ganiga C. Shivakumar, *et.al.* 2023, Analytical Study on Current Trends in the Clinico-Mycological Profile among Patients with Superficial Mycoses, *Journal of Clinical Medicine*, 2023; 12: p.3051.
35. Pillathil SJ, Natarajan S, Vinoth Kumar R, Murugesan S. The phytochemical potential of *Mimosa pudica* L. plants under enhanced solar UV-B (280-320 nm) radiation. *Appl Ecol Environ Sci.*, 2023; 11(4): p.130-134.
36. Komal Rizwan, *et.al.* 2022, Phytochemistry and Diverse Pharmacology of Genus *Mimosa*: A Review, *Biomolecules*, 2022; 1: p.283.

37. Suresh Mickymaray, *et.al.* 2020, Anti-Fungal Efficacy and Mechanisms of Flavonoids, Antibiotics, 2020; 9: 45.
38. Vijayalakshmi K, *et.al.* 2018, Antifungal Activity of *M. pudica* L. Against Selected Human Pathogens, International Journal of Advanced Scientific Research and Management, Oct 2018; 3(10).
39. Alamgir, *et.al* 2017- Therapeutic use of medicinal plants and their extracts: Pharmacognosy, vol 1.
40. Prosanta Pal, *et.al* 2015, Phytochemical Analysis of The Whole Plant of *Mimosa pudica* (Linn.), UJPSR, 2015; 1(1): p.1-9.
41. Ahmad H, Sehgal S, Mishra A, Gupta R, *Mimosa pudica* L.(Laajvanti): An overview, Pharmacognosy reviews, 2012; 6(12): p.115-124.
42. Havaladar VJ, Jadhav NY, Mali KK, Mali SS, Shinde SS, An overview on *Mimosa pudica* (touch-me-not plant), International Journal of modern Pharmaceutical research, 2022; 6(4): p.28-34.
43. Gupta S, Kumar R, Patel M, Singh P, *Mimosa pudica* L.: A sensitive, creeping, annual or perennial herb, A Review, International Journal of Phytopharmacology, 2021; 12(3): p.45-52.
44. Achummantakath H, Pramod L, Silpa M, *Mimosa pudica* L. – A sensitive plant: A Review, Journal of Pharmacognosy & Phytochemistry, 2019; 15: p.61-65.
45. N. S. Adikari. Prevention of disease through cure, Yojana, 2003; 36(1-3): p.42-44.
46. Tamilarasi T. and Ananthi T. Phytochemical analysis and anti-microbial activity of *Mimosa pudica* Linn. Research Journal of Chemical Sciences, 2012; 2(2): p.72-74.
47. Mei A, Kumarasinghe P, Raby E, Ricciardo B, Plant-based Therapies for dermatophyte infections, Tasman medical journal, 2022; 4(3): p.21-37.
48. Krishnamurthy V, Rajangam U, Antifungal Activity of *M. pudica* L. Against Selected Human Pathogens, International Journal of Advanced Scientific research & Management, 2018; 3(10): 79-87.
49. Ganesh P, Bijay K, Subrat K, Effects of *Mimosa pudica* L. leaves extract on anxiety, depression and memory, Avicenna Journal of Phytomedicine, 2016; 6(6): 696-710.
50. Khamsah S, Mahadeva R, Muhammad A, Phytochemical Screening, Total Flavonoid and Phenolic content Assays of various solvent extracts of Tepal of *Musa paradisiaca*, Malaysian Journal of Analytical Science, 2016; 20(5): p.1181-1190.
51. William Charles Evans, Trease & Evans Pharmacognosy, 16th Edition.

52. Bhagwat G, Raut A, Yadav S, Thin layer chromatographic analysis of Flavonoid in Mulberry leaf extract, International journal of research in applied science & engineering technology, 2023; 11(6): p.1227-1230.
53. Cai W., Gu X., Tang J. Extraction, purification, and characterisation of the flavonoids from *Opuntia milpa alta* skin". Czech J. Food Sci., 2010; 28: p.108–116.
54. Yahaya Mobmi Musa. Isolation and Purification of Flavonoids from the leaves of locally Produced *Carica papaya*. International Journal Of Scientific and Technology Research, 2015; 4: p.282-284.
55. Gharge S, Hiremath SI, Jivaje K, Kagawad P, Suryawanshi S, Quality control & Standardization quercetin in herbal medicines by spectroscopic & Chromatographic techniques, Future journal of Pharmaceutical sciences, 2021; 7: 176:1-12. Available from- <https://doi.org/10.1186/s43094-021-00327-y>
56. .Bijauliya RK, Kannoja P, Mishra P and Pathak GK, Isolation and Structure Elucidation of Quercetin like Structure from *Dalbergia sissoo* (Fabaceae), Journal of Drug Delivery and Therapeutics, 2020; 10(3-s): p.6-11.
57. Arora S, Itankar P, Extraction, isolation and identification of flavonoid from *Chenopodium album* aerial parts, Journal of traditional and complementary medicine, 2018; 8: p.476-482.
- 58.