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PHYTOCHEMICAL ANALYSIS OF THE WHOLE PLANT OF *Mimosa pudica* (Linn.)

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Abstract
Mimosa pudica (Linn.) 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. As the whole plant being used to cure various diseases so we have extracted all the plant constituents from whole plant powder of Mimosa pudica (Linn.) using 50% ethanol following cold maceration technique. Preliminary phytochemical evaluation showed the presence ofcarbohydrates, alkaloids, proteins, amino acids, tannins, phenolics, flavonoids, steroids, fixed oil, mucilage and saponins. Among these compounds alkaloid was found to exhibit different pharmacological properties. So later we have separated and identified the alkaloids present in the 50% ethanolic whole plant extract and its fractions.

Key words
Mimosa pudica (Linn.), phytochemical, alkaloids.

INTRODUCTION

Chemical diversity in natural product is an immensely rich source of new pharmaceuticals, cosmetics, agrochemicals and other economically important chemicals. Therapeutic potentials of herbal drug ranges from parts of plants, through simple extracts to isolated active constituents. Phytochemical evaluation compresses of different chemical tests and chemical assay. The isolation, purification and identification of active constituents are chemical methods of evaluation. The phytochemical evaluation also covers phytochemical screening carried out for establishing chemical profile of crude drug. The purity of crude drugs is ascertained by quantitative estimation of active chemical constituents present in them. The method may be useful in determining single active constituents or the group of related constituents present in the same drug. The spectrophotometric analysis using UV/ visible/ IR/ Fluorescence/ NMR/ Mass/ X-ray diffraction are physical methods of assay. The modern techniques like HPTLC, HPLC, GC, LCMS, GCMS are becoming popular in the field of molecular research, because:

- Most versatile, safest, dependable, efficient, fast test and sensitive chromatogram.
- Improved data processing capabilities.
- Identification, quantification finger printing of drug molecule.
- Detection of adulterant [1,2,3].

MATERIALS AND METHODS

Plant material

The plant *Mimosa pudica* (Linn.) grows nearly throughout the tropical and sub-tropical parts of India. It is common in waste ground, particularly where the climate is moist and warm.

The plant material was collected from Coimbatore district. It was identified and confirmed by the department of Pharmacogonosy, J.S.S. College of Pharmacy, Ootacamund, Nilgiris, Tamilnadu, India.

Treatment

The collected plant material was dried in shade and the dried material was coarsely powdered my means of mechanical grinder. The resulting powdered material was used for further studies [43].

Ash value: The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Ash value is a criteria to judge the identity or purity of crude drugs. Total ash usually consists of carbonates, phosphates, silicates and silica. In sulphated ash, all the oxides and carbonates are converted to sulphates. Acid insoluble ash usually consists mainly of silica,

Determination of total ash

About 3gm of the powdered drug was accurately weighed in a silica crucible which was previously ignited and weighed. The powdered drug was spreaded as a fine even layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450^{o}C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to the constant weight. The percentage of the total ash was calculated with reference to the air dried drug. The total ash value of whole plant of *Mimosa pudica* (Linn.) is recorded in Table No1.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25ml of 2 (M) hydrochloric acid for five minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible, was ignited, cooled and weighed. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug. The results are recorded in Table No1.

Determination of sulphated ash

A silica crucible was heated to redness for 10 minutes, allowed to cool in a desiccator and weighed. About 19m of the powdered drug was accurately weighed an taken in the above crucible. The crucible was ignited, gently at first until the drug was thoroughly charred. The crucible was cooled and the residue was moistened with 1ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800^{o}C :I: 25°C until all black particles had disappeared. The ignition was conducted in place protected from air currents. The crucible was allowed to cool, few drops of sulphuric acid was added and again heated. The ignition was carried out as before, allowed to cool and weighed. The operation was repeated until two successive weighing did not differ by more than 0.5mg. The percentage of sulphated ash was calculated with reference to the air dried drug and recorded in Table No.1.

Extractive value

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into the consideration, the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives.

Water soluble extractives: This method is applied to a drug which contains water soluble active constituents of crude drugs such as, tannins, sugars, plant acids, mucilage, glycoside, etc.

Alcohol soluble extractives

This method is frequently employed to determine the approximate resin content of drug.

i. Determination of water soluble extractives

Macerated 5g of the air dried coarse powder of whole plant of *Mimosa pudica* (Linn.) with 100ml of chloroform water in a closed flask for 24hours. Shaking frequently during the first 6hours and allowing to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish, dried at 105^{o}C and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried drug. The water soluble extractive value of the whole plant of *Mimosa pudica* (Linn.) is recorded in Table No.2.

ii. Determination of ethanol soluble extractives

Macerated 5gm of the air dried coarse powder of whole plant of *Mimosa pudica* (Linn.) with 100ml of 95% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25ml of the filtrate to dryness in a tared flat bottom shallow dish, dried at 105oC and

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weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug. The ethanol soluble extractive value of the whole plant is recorded in Table No.2

Preparation of extracts

About 700gms of powdered whole plant of *Mimosa pudica* (Linn.) was taken in a 2000ml of round bottom flask. It was first defated with petroleum ether and then cold extracted for 12 days using ethanol (50%) with occasional starring. The ethanolic extract was filtered through Whatmann filter paper to remove impurities present. The ethanolic extract was concentrated by rotary evaporator and finally placed in a desiccator to remove the excessive moisture [4].

Qualitative phytochemical analysis

The extracts of *Mimosa pudica* (Linn.) was subjected to the following chemical tests for the identification of various active constituents.

A. Tests for carbohydrates:

Molisch test

To 2 ml of the extract, add I ml of α-naphthol solution, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's test

To 1 ml of the extract, add equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's test: To 5 ml of Benedict's reagent, add 1 ml of extract solution and boil for 2 minutes and cool. Formation of a red precipitate shows the presence of carbohydrates.

B. Tests for alkaloids

Dragendorffs test

To 1 ml of the extract, add 1 ml Dragendorffs reagent, an orange red precipitate indicates the presence of alkaloids.

Wagner's test

To 1 ml of the extract, add 2 ml of Wagner's reagent, the formation of a reddish brown precipitate indicates the presence of alkaloids.

Mayer's test

To I ml of the extract, add 2 ml of Mayer's reagent, a dull white precipitate reveals the presence of alkaloids.

Hager's test

To I ml of the extract, add 3 ml of Hager's reagent, the formation of yellow precipitate confirms the presence of alkaloids.

Tests for proteins and amino acids

Biuret test

To 1ml of the extract add 1ml of 40% sodium hydroxide solution and 2 drops of I % copper sulphate solution. Formation of violet colour indicates the presence of proteins.

Xanthoprotein test

To 1ml of the extract add I ml of concentrated nitric acid. A white precipitate is formed, it is boiled and cooled. Then, 20% of sodium hydroxide or ammonia is added. Orange colour indicates the presence of aromatic amino acids.

Lead Acetate test

To the extract, I ml of lead acetate solution is added. Formation of a white precipitate indicates the presence of proteins.

Ninhydrin test

Add two drops of freshly prepared 0.2% ninhydrin reagent to the extract solution and heat. Development of blue colour reveals the presence of proteins, peptides or amino acids.

C. Tests for tannins and phenolics

To I ml of the extract, add ferric chloride, formation of a dark blue or greenish black colour product shows the presence of tannins.

To the extract, add potassium dichromate solution, formation of a precipitate shows the presence of tannins and phenolics.

D. Test for flavonoids

Shinoda Test

To 1 ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid formation of red colour shows the presence of flavonoids.

E. Test for triterpenoids

Dissolve two or three granules of tin metal in 2 ml thionyl chloride solution. Then, add 1 ml of the extract into the test tube. The formation of a pink colour indicates the presence of triterpenoids.

F. Tests for steroids

Libermann Burchard test

Dissolve the extract in 2 ml of chloroform in a dry test tube. Add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green, indicates the presence of steroids.

Salkowski test

Dissolve the extract in chloroform and add equal volumes of concentrated sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represents the steroid components in the tested extract. Liebermann's reaction: Mix 3ml – extract with 3ml acetic anhydride,

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heat and cool, add few drops of concentrated sulphuric acid, blue colour appears

G. Test for saponins

About 1 ml of extract is diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins.

H. Tests for fixed oils

Spot test: Press a small quantity of extract between two filter papers. Oil stains on paper indicate the presence of fixed oils.

Saponification test

To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization indicates the presence of fixed oils.

I. Tests for glycosides:

Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

Baljet test

To 1 ml of the test extract add I ml sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

Borntrager's test

Add a few ml of dilute sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer is treated with I ml of ammonia. The formation of red colour shows the presence of anthraquinone glycosides.

Keller Kiliani test

Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of glycosides.

J. Test for gums

Hydrolyse the test solution using dilute HCl. Perform Fehling's or Benedict's test. Red colour is developed.

K. Test for mucilages

Powdered drug material shows red colour with ruthenium red.

Powdered drug swells in water.

Fluorescence analysis: The organic molecules light usually over a specific range of wavelength, and many of them re-emit such radiations. This phenomenon is called as Luminescence. When the reemission of the absorbed light lasts only whilst the substance is receiving the exciting rays, the phenomenon is defined as fluorescence [5].

Chromatographic studies

a. Thin Layer Chromatography (TLC)
b. High performance Thin Layer Chromatography
c. Thin Layer Chromatography (TLC):

The TLC identity tests provided in the chromatographs include identification of the drug based on its major chemical constituents.

TLC of Alkaloids

Preparation of Extracts

Preparation of mother extract

1 gm of 50% ethanolic extract of whole plant of *Mimosa pudica* (Linn.) was dissolved in 50 ml of 50% ethanol by shaking for 15minutes at 60^{o}C with 50ml of 50% ethanol. It was filtered through Whattmann filter paper, and excess of ethanol was added into the marc. The resultant filtrate was made upto 100 ml with 50% ethanol to obtain a concentration of 10mg/ml.

Preparation of alkaloidal fraction

1 gm of 50% ethanolic extract of *Mimosa pudica* (Linn.) (whole plant) was moistened with 1ml of 10% ammonia solution, and then extracted by shaking for 15minutes at 60^{o}C with 5ml of methanol. It was filtered through Whatmann filter paper, and excess of methanol was added into the marc. The resultant filtrate was made up to 100 ml with methanol to obtain a concentration of 10mg/ml.

Preparation of methanolic fraction

1 gm of 50% ethanolic extract of whole plant of *Mimosa pudica* (Linn.) was dissolved in 50 ml of methanol by shaking for 15minutes at 60^{o}C with 50ml of methanol. It was filtered through Whattmann filter paper, and excess of ethanol was added into the marc. The resultant filtrate was made upto 100 ml with methanol to obtain a concentration of 10mg/ml.

Preparation of ethyl acetate fraction

1 gm of 50% ethanolic extract of whole plant of *Mimosa pudica* (Linn.) was dissolved in 50 ml of ethyl acetate by shaking for 15minutes at 60^{o}C with 50ml of ethyl acetate. It was filtered through Whattmann filter paper, and excess of ethyl acetate was added into the marc. The resultant filtrate was made upto 100 ml with ethyl acetate to obtain a concentration of 10mg/ml.

Stationary phase- Silica gel GF $_{254}$ plate

Mobile phase

Chromatography was tried with the following solvent systems:

- Toluene-ethylacetate-dimethyl amine (70:20:10)
- Chloroform-dimethyl amine (90:20)

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- Toulene-acetone-ethanol-Conc.ammonia (40:40:6:2)
- Acetone-water-Conc.ammonia (90:7:3)
- Ethyl acetate-methanol-water (100:13.5:10)
- Toulene-choroform-ethanol (28.5:57:14.5)
- N-Heptane-ethylmethylketone-methanol (58:34:8)
- Chloroform-methanol (85:15)
- Toulene-methanol (86:14)
- N-Propanol-formin acid-water (90:1:9)
- Cyclohexane – chloroform – glacial acetic acid (45:45:10)
- Acetonitrile-methanol (4:6)

The solvent system, Acetonitrile-methanol in the ratio 4:6, gave more satisfactory spot for alkaloid.

Detection of component

Visualising agent: Dragendorff's reagent
Colour of the spot: Reddish brown
Application of the extract
All the extract 10 mg/ml was taken in a capillary tube and spotted on the plate keeping a distance of about 2 cm above the base of the plate.

Development of the chromatogram

The plates were then developed in TLC chamber previously provided and saturated with appropriate solvent system. After the development of the chromatogram, the plates were removed treated with visualizing agents and examined for the presence of the different spots. The R_f value were calculated and tabulated in table no.6

b. High Performance Thin Layer Chromatography (HPTLC)

Recent developments in the practice of thin-layer chromatography have resulted in a breakthrough in performance which has led to the expression 'high performance thin-layer chromatography. These developments have not been the result of any specific advance in instrumentation (as with HPLC), but rather the culmination of improvements in the various operations involved in TLC. The three chief features of HPTLC are summarised below:

Quality of the adsorbent layer

Layers for HPTLC are prepared using specially purified silica gel with average particle diameter of 5-15µm and a narrow particle size distribution. The silica gel may be modified if necessary, e.g. chemically bonded layers are available commercially as reverse-phase plates. Layers prepared using these improved adsorbents give up to about 5000 theoretical plates and so provide a much improved performance over conventional TLC; this enables more difficult separations to be effected using HPTLC, and also enables separations to be achieved in much shorter times.

Methods of sample application

Due to the lower sample capacity of the HPTLC layer, the amount of sample applied to the layer is reduced. Typical sample volumes are 100-200nL which give starting spots of only 1.0-1.5mm diameter; after developing the plate for a distance of 3-6 cm, compact separated spots are obtained giving detection limits about ten time~ better than in conventional TLC. A further advantage is that the compact starting spots allow an increase in the number of samples which may be applied to the HPTLC plate.

The introduction of the sample into the adsorbent layer is a critical process in HPTLC. For most quantitative work a platinum-iridium capillary of fixed volume (100 or 200 nL), sealed into a glass support capillary of larger bore,' provides a convenient spotting device. The capillary tip is polished to provide a smooth, planar surface of small area *(ca* 0.05 mm2), which when used with a mechanical applicator minimises damage to the surface of the plate; spotting by manual procedures invariably damages the surface.

The availability of scanning densitometers

Commercial instruments for *in-situ;* quantitative analysis based on direct photometric measurement have played an important role in modern thin layer chromatography. Although double beam instruments are available, single beam single wavelength operation is mainly used in HPTLC since the quality and surface homogeneity of the plates are generally very good. High performance thin-layer chromatography has found its greatest application in the areas of clinical (e.g. analysis of drugs in blood), environmental and quantitative phytochemical analysis.

Types of samples applied

Mother extract: 10 mg/ml, Alkaloidal fraction: 10 mg/ml, Methanolic fraction: 10 mg/ml,
Ethyl acetate fraction: 10 mg/ml
Mobile phase: Acetonitrile : methanol (4:6).Stationary phase: Silica gel GF_{254}
Detection of component:
Detector: 254 nm
Source : Deuterium [6,7]

RESULT AND DISCUSSION

The plant *Mimosa pudica* (Linn.), belonging to the family leguminosae, it is widely grown plant throughout the tropical and subtropical parts of India. In order to standardize the raw materials, the various physicochemical constants like ash values and extractive values were carried out. The results are shown in table No.1 and table No.2.

Table 1: Ash values of whole plant of *Mimosa pudica* (Linn.)

S.No.	Type of ash	Percentage (W/W)
1	Total Ash	5.322 % W/W
2	Acid Insoluble Ash	1.529 % W/W
3	Sulphated Ash	8.6196 % W/W

Table 2: Extractive values of whole plant of *Mimosa pudica* (Linn.)

S.No.	Type of Extractive Value	Percentage
1	Alcohol	23.867%W/W
2	Water	19.717%W/W

The 50% ethanolic extract was subjected to preliminary phytochemical tests to find out the type of active constituents present and the results are shown in table No.3

Table 3: Data showing the nature of the phytoconstituents present in *Mimosa pudica* (Linn.)

Phytoconstituents	Observation
Carbohydrates	+
Alkaloids	+
Proteins & Amino acids	+
Tannins & Phenolics	+
Flavonoids	+
Triterpenoids	-
Steroids	+
Glycosides	-
Fixed oils	+
Gums	-
Mucilages & Saponins	+

(+) Indicates the presence of chemical constituents
(-) Indicates the absence of chemical constituents

Different sample like leaf powder, stem powder, whole plant powder, 50% methanolic extract and 50% ethanolic extract of the whole plant were examined under day light and UV light to find out the the presence of any fluorescent compound within them. The results are shown in table No.4

Table 4: Data showing the Fluorescence analysis of powder and whole plant extract of *Mimosa pudica* (Linn.)

Sample	Reagent used	Visible	UV
Mimosa pudica(Linn.) Leaf powder	1. 1(N) NaOH 2. 1(N) NaOH in Alcohol 3. 1(N) HCl	Yellowish green Green Gray Orange	Green Green Green Green

	4. 50% HNO3		
Mimosa pudica(Linn.) Stem powder	1. 1(N) NaOH 2. 1(N) NaOH in Alcohol 3. 1(N) HCl 4. 50% HNO3	Yellowish green Yellowish green Light brown Orange	Green Green Green Green
Mimosa pudica (Linn.)Powder (Whole plant)	--	Yellowish gray	Green
50%methanolic extract of *Mimosa pudica* (Linn.) (Whole plant)	--	Deep green	Green
50% ethanolic extract of *Mimosa pudica* (Linn.) (Whole plant)	1. 1(N) NaOH 2. 1(N) NaOH in Alcohol 3. 1(N) HCl 4. 50% HNO3	Yellowish brown Yellowish brown Light brown Orange	Green Green Green Green

The different extracts of the whole plant were subjected to TLC analysis for alkaloids and the results are shown in table No.5

Table 5: Data showing the R_f values of the TLC studies on the 50% ethanolic extract of *Mimosa pudica* (Linn.)

S. No.	Extract(10 mg/ml)	Solvent System	TLC study for	R_f values
1	Mother extract	Acetonitrile:methanol (4:6)	Alkaloid	0.51
2	Alkaloidal fraction	Acetonitrile: methanol (4:6)	Alkaloid	0.52 , 0.71
3	Methanolic fraction	Acetonitrile: methanol (4:6)	Alkaloid	0.50
4	Ethyl acetate fraction	Acetonitrile: methanol (4:6)	Alkaloid	--

The different types of extract were subjected to HPTLC studies. The results are shown in table No.6

Table 6: Date showing HPTLC of alkaloids in different samples of *Mimosa pudica* (Linn.) 50% ethanolic extract.

S. No.	Sample	Quantity of sample applied	Scanning wavelength	R_f value
1	Mother extract	10 µl	254 nm	0.04, 0.25, 0.45, 0.54, 0.69
2	Alkaloidal fraction	10 µl	254 nm	0.05, 0.17, 0.22, 0.27, 0.37, 0.42, 0.51, 0.73, 0.82
3	Methanolic fraction	10 µl	254 nm	0.06, 0.13, 0.18, 0.28,0.44 0.50, 0.73,0.82
4	Ethyl acetate fraction	10 µl	254 nm	--

Fig.1: HPTLC of alkaloids in mother extract

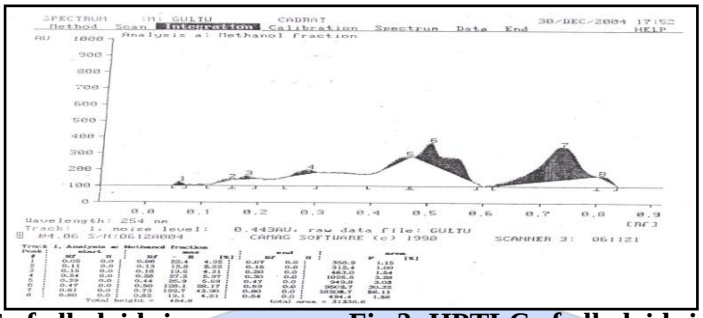


Fig.2: HPTLC of alkaloids in methanolic fraction

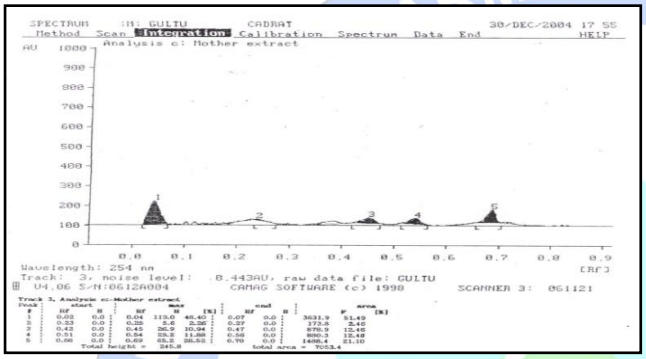
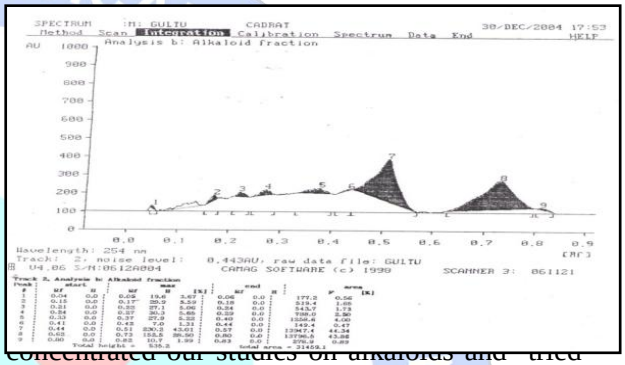


Fig.3: HPTLC of alkaloids in alkaloidal fraction



Phytochemical analysis conducted on the 50% ethanolic extract of the whole plant revealed the presence of several active constituents which are known to exhibit medicinal as well as physiological activities . Analysis of the plant extracts revealed the presence of phytochemicals such as carbohydrates, alkaloids, proteins, amino acids, tannins, phenolics, flavonoids, steroids, fixed oils, mucilage and saponins. The alkaloids are one of the largest and most ubiquitous groups of plant metabolites. They have been associated with medicinal uses for centuries and

one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids [8]. So, we have concentrated our studies on alkaloids and tried to separate and identify the alkaloids by TLC and HPTLC techniques. The TLC studies revealed that the mother extract and the methanolic fraction contains alkaloids with R_f values 0.51 and 0.50 respectively but

ethyl acetate fraction was devoid of any alkaloid. The results were further confirmed by HPTLC technique.

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. 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.

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