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Research Article

HPTLC Identification, Quantification and Validation of p-Coumaric Acid and Quercetin in Aqueous Extract of *Mimosa pudica*.

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ABSTRACT
The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. *Mimosa pudica* also has been reported to possess antinociceptive, antihyperglycemic, antivenom, immunomodulatory, anticonvulsant, antihepatotoxic, antifertility, diuretic and wound healing activity. These pharmacological studies have established a scientific basis for therapeutic uses of this plant. In our study attempts have been made to develop new HPTLC method for identification, quantification and validation of p-coumaric acid and quercetin in aqueous extract of*Mimosapudica.*

Key words-*Mimosa pudica*,HPTLC,validation

INTRODUCTION

*Mimosapudica*is commonly known as*Lajjalu.* This plant usually grows as a weed in fields or is cultivated as a garden plant. *Lajjalu*consist of dried whole plant of *Mimosa pudica Linn. (Fam. Fabaceae)*.The plant is a diffused undershrub, sensitive to touch, and 25-50cm in height[1].*Mimosa pudica* is native to south and central America and is also cultivated in India[1,4].*Mimosa pudica*isknown as a sensitive plant due to rapid movement of leaves in response to physical and chemical stimuli. These movements are controlled by biological clock and are periodic(circadian rhythm) in nature, which are called as nyctinastic movements[1].Based on sensitivity on *Mimosa pudica* , Sanberg attempted to correlate animal system and neural capacity of plant[2].It is reported to be useful in the treatment of diarrhea (athisaara)Amoebic dysentery(raktaatisaara), bleedingpiles, and to arrests bleeding .Literature survey reveals that various extracts of *Mimosa pudica*when subjected to pharmacological studies,were found to be effective as antinociceptive, antihyperglycemic, antivenom, immunomodulatory, anticonvulsant, antihepatotoxic, antifertility, diuretic etc[4-18]

MATERIALS AND METHODS

Formic acid (AR),ethyl acetate(AR), toluene(AR), p-coumaric acid(EP), quercetin(EP) were purchased from Merck and Sigma alderich.

Plant material

The plant Mimosa pudica(MP) was collected from garden at Badlapurand authenticated at Blatter herbarium St.Xavier's college Mumbai, which matches with Blatter herbarium specimen number (JF 1523).

Preparation of plant extract

The plant MP was dried under shade at room temperature. Then leaves were separated, powdered and passed through sieve no-#40 mechanically. Dried leaves were extracted in Soxhlet apparatus by using 70% ethanol (MPHA) and water (MPAQ) as solvents. Extracts were air dried .The dry extracts were stored in an air- tight container in refrigerator (5^0 +/- 1^0 c) for experimental use.[19]

Identification, Quantification and Validation

High performance thin layer chromatography of the MPHA and MPAQ extract of leaves of Mimosa pudica was carried out for identification, quantification and validation of quercetin and p-coumaric acid present in plant extracts MPHA and MPAQ.

Preparation of standard and test solutions

Quercetin, p-coumaric acid and MPHA extract were dissolved in methanol and MPAQ extract was dissolved in distilled water to prepare following concentrations.

Extracts	Concentration (stock solution)	Standards	Concentration (stock solution)
MPHA extract	0.0579 mg/ml	Quercetin	1mg/ ml
MPAQ extract	0.0513 mg/ml	p-coumaric acid	1 mg/ml

HPTLC method

Quantification of p-coumaric acid and quercetin in MPAQ extract and validation of method was performed using HPTLC method. After preparation of standard and extract solutions, solution were applied over the pre-coated silica gel 60 F254 plates (as a stationary phase) usingCAMAG HPTLC SYSTEM 100µl Hamilton syringe with the help of Linomat V applicator, application distance maintained at 5mm. Mobile phase was Toluene: Ethyl acetate: Formic

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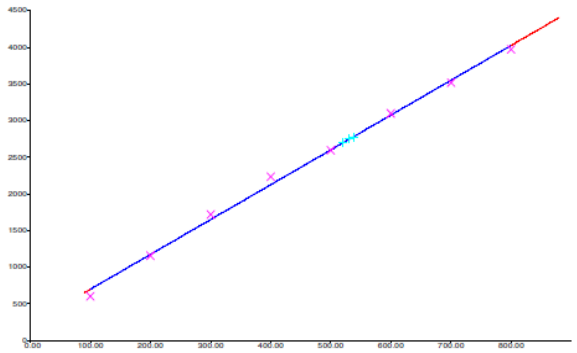

Fig.1 Calibration curve for p-coumaric acid

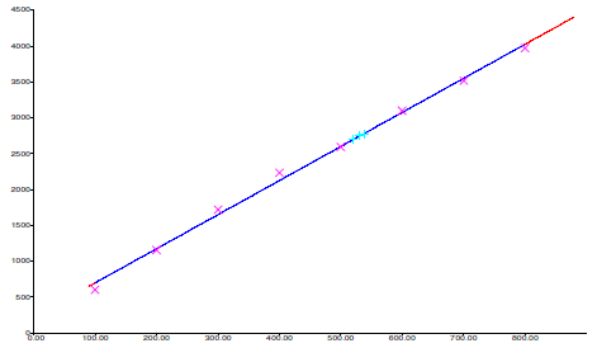

Fig.2 Calibration curve for quercetin

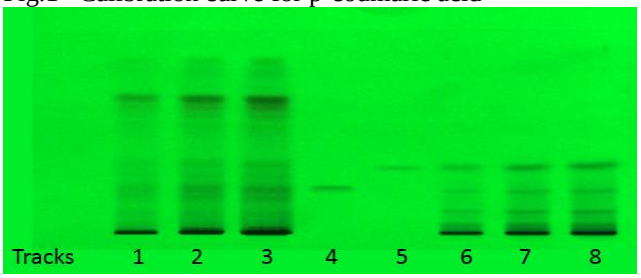

Fig.3 Identification of p-coumaric acid and quercetin in MPAQ extract

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	Hydro alcoholic
2	Sample	1	Hydro alcoholic
3	Sample	1	Hydro alcoholic
4	Sample	3	Quercetin
5	Sample	4	p-Coumaric acid
6	Sample	2	Aqueous
7	Sample	2	Aqueous
8	Sample	2	Aqueous

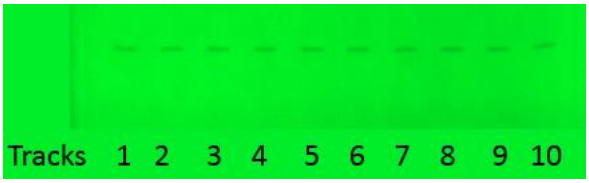

Fig.5 Precision (interday) p-coumaric acid

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	STANDARD P-COU
2	Sample	1	STANDARD P-COU
3	Sample	1	STANDARD P-COU
4	Sample	1	STANDARD P-COU
5	Sample	1	STANDARD P-COU
6	Sample	1	STANDARD P-COU
7	Sample	1	STANDARD P-COU
8	Sample	1	STANDARD P-COU
9	Sample	1	STANDARD P-COU
10	Sample	1	STANDARD P-COU

acid with the ration of (8:2:0.5).Plate was placed into CAMAG twin through chamber for saturation for 15 min. After drying plates were scanned using CAMAG TLCscanner, 170422 at 3 different wavelengths 254,366,540 nm. Integrator software win CATS used for calculation[20].

Parameters validated by HPTLC

1) Limit of detection-Lowest amount of analyte that could be detected on the basis of signal to noise ration. It can be determined by spotting concentrations of MPAQ on the plate.

2) Limit of quantitation-The lowest amount of p-coumaric acid and quercetin that could be quantitatively determined with definite precision and accuracy was calculated on the basis of signal to noise ratio.

3) Linearity/Range-The linearity of method was performed using standard solutions of p-coumaric acid and quercetin.

4) Precision-Precision is reported in terms of coefficient of variation over range of quantitation for single experiment in which standards were assayed in replicate (intraday) and in series of experiments in which standards were assayed over several experiments (interday).

Chromatographic conditions for HPTLC

Instrument	Camag Linomat V applicator
Stationary phase	HPTLC plates silica gel 60 F254 pre-coated plates (20×10 cm)
Chamber Saturation Time	10 min
Solvent Volume	20 ml
Developing Distance	80 mm
Detector	UV
Scanning Wavelengths	254,366,540 nm
Application Mode	CAMAG HPTLC SYSTEM 100µl Hamilton syringe
Development Mode	CAMAG twin through chamber
Developing Solvent	Toluene: Ethylacetate:Formic acid (8:2:0.5)
Application Bandwidth	5 mm
Developing Time	15 min
Scanner	CAMAG TLC scanner, 170422

RESULTS

Parameters validated by HPTLC method

Accuracy(% recovery)

P-coumaric acid	100.6 %
Quercetin	81.8%
CV(coeffcient of variation)	1.64
Precision(standatrd p-coumaric acid)	
Interday (%CV)	2.02%
Intraday(%CV)	1.56
Precision(standard quercetin)	
Interday (%CV)	2.36
Intraday(%CV)	2.12 %
Linearity (R^2)	0.998
LOD	0.1µg
LOQ	0.3µg
SD	3.05%

DISCUSSION

HPTLC is a modern chromatographic technique in which the principle of TLC is automated by which the samples are accurately and precisely estimated which can be utilized for both qualitative and quantitative purpose.In quantification studies content of p-coumaric acid was found to be 0.13% and content of quercetin was found to be 0.02% in *Mimosa pudica* aqueous extract. The accuracy (%recovery)was found to be 100.6% for p-coumaric acid and 81.8% for quercetin. For standard p-coumaric acid % CV was found to be 2.02% in interday precision, whereas %CV was found to be 1.56%in intraday precision. For standard quercetin% CV was found to be 2.36% ininterday precision&%CV was found to be2.12% in intraday precisionwhich represents excellent precision and reproducibility of the method. LOD and LOQ were found to be0.1µg and 0.3µg for both p-coumaric acid and quercetinwhich indicate the adequate sensitivity of the method.The linearity range was found to be 100-800 ng with R^2=0.998.

CONCLUSION

In the present investigation a novel solvent system for HPTLC analysis of *Mimosa pudica* was designed which provides sensitive, precise and reproducible method for determination of p-coumaric acid and quercetine in aqueous extract of *Mimosa pudica*, and thus can be used for quantitative studies.

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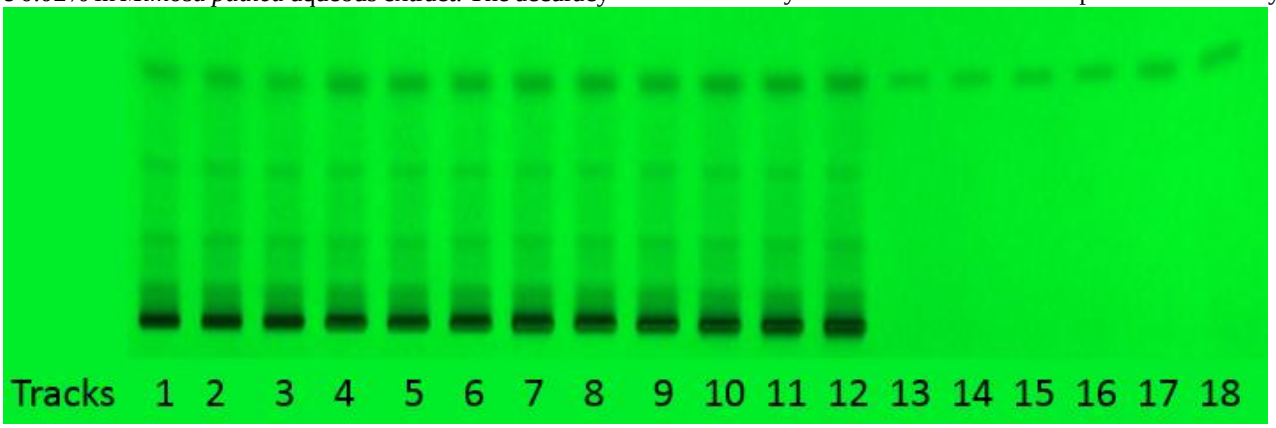

Fig.4 Accuracy (p-coumaric acid)

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	15.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>2	25.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>3	35.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>4	45.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>4*	45.0 mm	4.0 µl	2	P-COUMARIC	Yes
>5	55.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>5*	55.0 mm	4.0 µl	2	P-COUMARIC	Yes
>6	65.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>6*	65.0 mm	4.0 µl	2	P-COUMARIC	Yes
>7	75.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>7*	75.0 mm	5.0 µl	2	P-COUMARIC	Yes
>8	85.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>8*	85.0 mm	5.0 µl	2	P-COUMARIC	Yes
>9	95.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>9*	95.0 mm	5.0 µl	2	P-COUMARIC	Yes
>10	105.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>10*	105.0 mm	6.0 µl	2	P-COUMARIC	Yes
>11	115.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>11*	115.0 mm	6.0 µl	2	P-COUMARIC	Yes
>12	125.0 mm	5.0 µl	1	AQ EXTRACT	Yes
>12*	125.0 mm	6.0 µl	2	P-COUMARIC	Yes
>13	135.0 mm	4.0 µl	2	P-COUMARIC	Yes
>14	145.0 mm	4.0 µl	2	P-COUMARIC	Yes
>15	155.0 mm	5.0 µl	2	P-COUMARIC	Yes
>16	165.0 mm	5.0 µl	2	P-COUMARIC	Yes
>17	175.0 mm	6.0 µl	2	P-COUMARIC	Yes
>18	185.0 mm	6.0 µl	2	P-COUMARIC	Yes

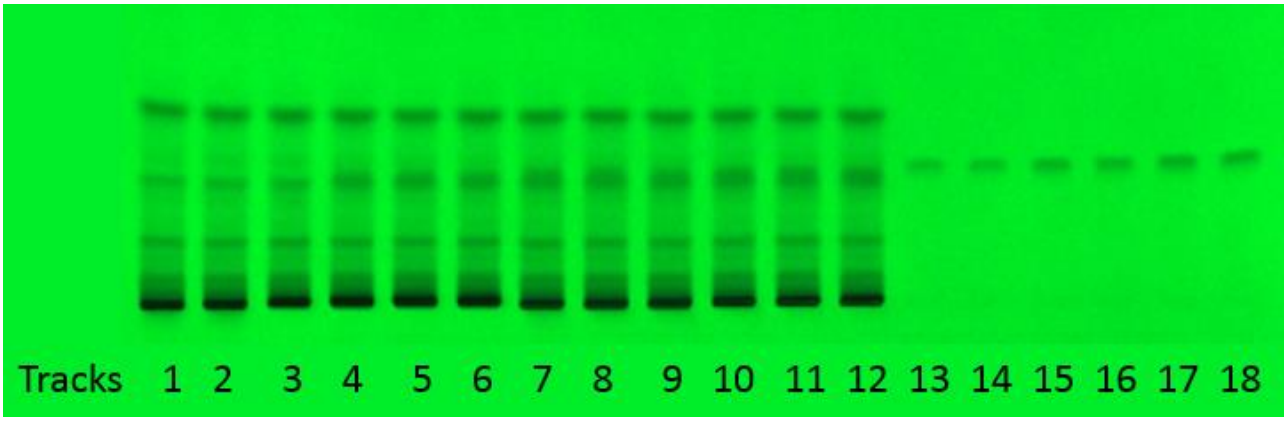

Fig.8 Accuracy (quercetin)

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	15.0 mm	15.0 µl	1	EXTRACT	Yes
>2	25.0 mm	15.0 µl	1	EXTRACT	Yes
>3	35.0 mm	15.0 µl	1	EXTRACT	Yes
>4	45.0 mm	15.0 µl	1	EXTRACT	Yes
>4*	45.0 mm	4.0 µl	2	QUERCETIN	Yes
>5	55.0 mm	15.0 µl	1	EXTRACT	Yes
>5*	55.0 mm	4.0 µl	2	QUERCETIN	Yes
>6	65.0 mm	15.0 µl	1	EXTRACT	Yes
>6*	65.0 mm	4.0 µl	2	QUERCETIN	Yes
>7	75.0 mm	15.0 µl	1	EXTRACT	Yes
>7*	75.0 mm	5.0 µl	2	QUERCETIN	Yes
>8	85.0 mm	15.0 µl	1	EXTRACT	Yes
>8*	85.0 mm	5.0 µl	2	QUERCETIN	Yes
>9	95.0 mm	15.0 µl	1	EXTRACT	Yes
>9*	95.0 mm	5.0 µl	2	QUERCETIN	Yes
>10	105.0 mm	15.0 µl	1	EXTRACT	Yes
>10*	105.0 mm	6.0 µl	2	QUERCETIN	Yes
>11	115.0 mm	15.0 µl	1	EXTRACT	Yes
>11*	115.0 mm	6.0 µl	2	QUERCETIN	Yes
>12	125.0 mm	15.0 µl	1	EXTRACT	Yes
>12*	125.0 mm	6.0 µl	2	QUERCETIN	Yes
>13	135.0 mm	4.0 µl	2	QUERCETIN	Yes
>14	145.0 mm	4.0 µl	2	QUERCETIN	Yes
>15	155.0 mm	5.0 µl	2	QUERCETIN	Yes
>16	165.0 mm	5.0 µl	2	QUERCETIN	Yes
>17	175.0 mm	6.0 µl	2	QUERCETIN	Yes
>18	185.0 mm	6.0 µl	2	QUERCETIN	Yes

Fig.6 Precision (intraday) p-coumaric acid

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	STANDARD P-COU
2	Sample	1	STANDARD P-COU
3	Sample	1	STANDARD P-COU
4	Sample	1	STANDARD P-COU
5	Sample	1	STANDARD P-COU
6	Sample	1	STANDARD P-COU
7	Sample	1	STANDARD P-COU
8	Sample	1	STANDARD P-COU
9	Sample	1	STANDARD P-COU
10	Sample	1	STANDARD P-COU

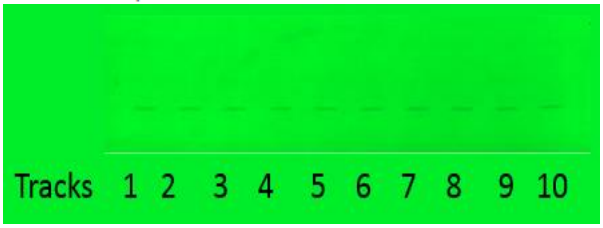

Fig.7 Linearity p-coumaric acid

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	STANDARD P-COU
2	Sample	1	STANDARD P-COU
3	Sample	1	STANDARD P-COU
4	Sample	1	STANDARD P-COU
5	Sample	1	STANDARD P-COU
6	Sample	1	STANDARD P-COU
7	Sample	1	STANDARD P-COU
8	Sample	1	STANDARD P-COU
9	Sample	1	STANDARD P-COU
10	Sample	1	STANDARD P-COU

Fig.9 Precision(interday) quercetin

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	Quercetin std
2	Sample	1	Quercetin std
3	Sample	1	Quercetin std
4	Sample	1	Quercetin std
5	Sample	1	Quercetin std
6	Sample	1	Quercetin std
7	Sample	1	Quercetin std
8	Sample	1	Quercetin std
9	Sample	1	Quercetin std
10	Sample	1	Quercetin std

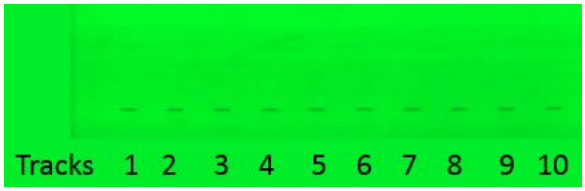

Fig.10 Precision(intraday) quercetin

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	Quercetin std
2	Sample	1	Quercetin std
3	Sample	1	Quercetin std
4	Sample	1	Quercetin std
5	Sample	1	Quercetin std
6	Sample	1	Quercetin std
7	Sample	1	Quercetin std
8	Sample	1	Quercetin std
9	Sample	1	Quercetin std
10	Sample	1	Quercetin std

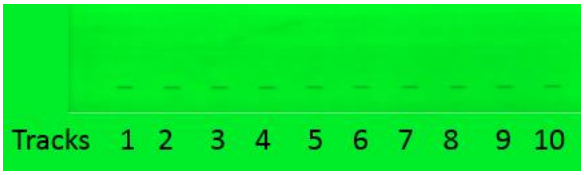

Fig.11 Linearity(quercetin)

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	Quercetin std
2	Sample	1	Quercetin std
3	Sample	1	Quercetin std
4	Sample	1	Quercetin std
5	Sample	1	Quercetin std
6	Sample	1	Quercetin std
7	Sample	1	Quercetin std
8	Sample	1	Quercetin std
9	Sample	1	Quercetin std
10	Sample	1	Quercetin std

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