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Research Article

IMPACT OF GEOGRAPHICAL VARIATION ON RUTIN CONTENT FROM *MIMOSA PUDICA* L. USING HPTLC TECHNIQUE

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ABSTRACT

Mimosa pudica L. (Mimosaceae) commonly known as touch-me-not is reported in treatment of menorrhagia, dysfunctional uterine bleeding, and urinary tract infections etc. It is reported for various pharmacological activities like antiulcer, anti-inflammatory, anticancer activity etc, due to the presence of various reported biomarkers like gallic acid, mimosine, mimosinic acid, betasitosterol etc. *Mimosa pudica* is also a part of various traditional formulations like *Samangadi Churna, Pushyanuga Churna* etc. In order to standardize the extract, phytochemical and proximate analysis of *Mimosa pudica* (whole plant) has been carried out along with its chromatographic evaluation. HPTLC fingerprint has been developed as a quality assurance tool. Further, a HPTLC method has been developed and validated as per ICH guidelines to quantitate the content of rutin from plant samples collected from various geographical regions of India. Extraction efficiencies of solvents of varying polarity and effect of various extraction techniques, on plant samples in terms of marker content were also analyzed. The concentration of phytochemical marker varied in samples collected from different regions. Variation was also observed in the samples extracted using solvents of varying polarity and different extraction techniques. The data generated in the current study can be used as a quality control tool for the use of authentic sample of *Mimosa pudica* singularly and as a part of various medicinal formulations. The method developed for the estimation of Rutin can be applied to various biological matrices. The variation in content of rutin implies the effect of regional and climatic variation on marker content.

Keywords: Geographical, HPTLC, *Mimosa pudica*, Rutin and Validation.

INTRODUCTION

In traditional system of herbal drugs are recommended in treatment of various disorders (Kamboj, 2000). These crude drugs contain biological active constituent that promote health and alleviate illness. Many folk practioners prescribe herbal drugs in treatment of various disorders. *Mimosa pudica* L. (Mimosaceae) is one such traditionally important undershrub which is used for treatment of various ailments like dysentery, leprosy, vaginal and uterine complaints, urinary infections since ages and is used as one of the important ingredients in prescription of folk practioners and also in various traditional formulations like *Samangadi Churna, Pushyanuga Churna, Gangadhar Churna, Kutajavaleha* etc which are reported for gynecological disorders, piles, diarrhea etc. The plant is known by different vernacular names such as *samanga* (Sanskrit), *lajjalu* (Marathi) etc. *Mimosa pudica* (whole plant) is also described in the Ayurvedic pharmacopoeia and Quality of Indian medicinal plants for its therapeutic

attribution (Ayurvedic Pharmacopoeia of India and Quality standards of Indian medicinal plants, 2011). Apart from traditional knowledge *Mimosa pudica* is scientifically reported for various pharmacological activities like anti diabetic, antioxidant, anti carcinogenic, wound healing, female reproductive problems etc due to the presence of several classes of secondary biologically active metabolites like tannins, glycosides, alkaloids, flavonoids, phenols, betasitosterol, gallic acid, mimosine, norepinephrine, mimosinic acid etc (Shaikh et al., 2016; Subramanian, et al., 2015; Joseph et al., 2013; Jadhav, 2012; Tamilarasi and Ananthi, 2012; Azmi et al., 2011).

Concentration of these secondary metabolites and other biochemical markers responsible for various pharmacological activities in plant tend to change due to environmental factors, due to which the quantitative estimation of marker based compounds and other major constituents, is a major challenge. Thus exact time and method of harvesting is essential in order to maintain its therapeutic potency (Dharmendra et al., 2012; Kamboj, 2000).

As there is an increasing demand for polymers of natural origin in addition to the active pharmaceutical ingredient in various pharmaceutical formulations. These formulations are deliberately substituted with other low quality material for commercialization (Sharma et al., 2010). Hence, standardization and use of authentic plant material is an integral step for the establishment of a consistent biological activity and to maintain the chemical profile of herbal drugs (Choudhary and Sekhon, 2011).

Thus the aim of the study is to standardize *M. pudica* in terms of physicochemical parameters, phytochemical and chromatographic evaluation. Due to varied application of these secondary active metabolites, quality of *M. pudica* was evaluated using one such biologically active biomarker Rutin, a flavonoid glycoside reported for anti-inflammatory, antihepatotoxic, antiulcer, anti cancer, anti-oxidant and reduce low density lipoprotein (LDL) oxidation (Ganeshpurkar and Saluja, 2017; Naif et al., 2015; Ahmed and Rao, 2013) using validated chromatographic technique. The data generated in the current study can be used as a quality control tool for the use of authentic sample of *Mimosa pudica* which is one of the key ingredients in many herbal formulations.

MATERIALS AND METHOD

Plant material

The whole plant of *Mimosa pudica* was collected and authenticated by Agharkar Research Institute, Pune (Authentication No. ARI 10-75) and a voucher specimen was deposited for further reference. Samples were shade dried for 7 days, then dried at 37±2ºC, powdered in a mixer grinder, sieved through 85mesh (BSS) and stored in air-tight containers at room temperatures.

Reference standards and chemicals

Rutin (≥95% purity, Figure 1) was procured from Sigma aldrich. All the chemicals used were of analytical grade and were procured from Merck speciality Pvt Ltd. Mumbai, India

Preparation of standard solution

10.0 mg of standard (rutin) was accurately weighed and transferred to 10.0 mL standard volumetric flask. The content was initially dissolved in minimum quantity of methanol, sonicated and then diluted up to the mark with methanol. The stock solution of 1000.0µg/mL was used to prepare working solutions of 100.0µg/mL, 10.0µg/mL and 1.0 µg/mL.

Physicochemical and Phytochemical evaluation

The physicochemical parameters of the *M. pudica* (whole plant) such as foreign organic matter, loss on drying, ash content (total, acid insoluble and water soluble) and extractive values were determined using standard pharmacopoeial methods (Indian pharmacopoeia, 2010; Khandelwal, 2008; Jadhav, 2012 and Nair, 2007). Similarly the qualitative phytochemical screening of some major class of secondary metabolites (flavonoids, tannins, glycosides, alkaloids and resins) was carried out by performing preliminary phytochemical test as per the reported method (Khandelwal, 2008).

Extraction of *M. pudica* (whole plant) for phytochemical screening

The powder sample (1.0 g) was extracted with ethanol / water (10.0 mL), vortex mixed for a minute and kept standing for overnight extraction at room temperature followed by filtration through whatmann filter paper no. 1. The filtrate was subjected to phytochemical screening of secondary metabolites.

Optimization of extraction conditions for chromatographic technique

Extraction of *M. pudica* (whole plant) for phytochemical fingerprint

The powder sample (1.0 g) was extracted with ethanol (10.0 mL), vortex mixed for a minute and sonicated for 20 min and kept on shaker for overnight extraction for 6hrs followed by filtration through whatmann filter paper no. 1. The filtrate was subjected to HPTLC analysis for development of a phytochemical fingerprint and for separation and quantitation of rutin.

Optimized chromatographic conditions for phytochemical fingerprint and quantitation of rutin

Chromatographic separation of the phytochemical constituents was achieved on TLC plates (E. Merck) precoated with silica gel $60F_{254}$ (0.2 mm thickness) on aluminium sheet support. To develop HPTLC fingerprint of *M. pudica* (whole plant), the sample (10.0 µL) was applied to the plate as a band of 7.0 mm wide and at a distance of 12.0 mm from the edges. Each plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre saturated with the mobile phase for 20 min. After development, the plate was dried in a current of air at room temperature. The plate was derivatized using 1% Anisaldehyde Sulphuric reagent and dried in oven preset at 110 ^{o} C. All measurements were performed at 22 ± 1^{o}C. Plate was photo-documented at 254 nm (before derivatization), 366 nm and 550 nm (after derivitization, Figure 3).

To separate rutin from *M. pudica* (whole plant), the sample (10.0 µL) and rutin standard (100.0 µg/ mL) were spotted on TLC plates as bands of 7.0 mm wide and at a distance of 12.0 mm from the edges of similar instrumental conditions. The plate was developed up to a distance of 85.0mm in CAMAG twin trough glass chamber pre-saturated with mobile phase for 20min. The plate was scanned and photo-documented at 254nm.

METHOD VALIDATION

To validate the HPTLC method, a series of assays like determination of specificity, sensitivity, linearity, precision, recovery and assay were performed as per ICH guidelines. Further statistical evaluations were performed, analysis was performed in triplicate and the variations were evaluated in terms of mean, SD and % CV and % mean difference using Microsoft Excel.

ASSAY AND METHOD APPLICATION

The developed method was used to determine the content of rutin from samples of *M. pudica* collected from different geographical locations of India, using different extraction techniques and from different solvents. R_f and relative peak area from the samples of *M. pudica* related to the peak from rutin were calculated using regression equation. Microsoft Excel was used to determine mean, standard deviation, relative standard deviation and mean difference during the analysis.

Estimation of rutin from the whole plant of *M. pudica* using solvents of varying polarity

The method was applied to evaluate the content of rutin from the whole plant of *M. pudica* extracted in different solvents of varying polarity (hexane, toluene, ethyl acetate, methanol, hydroalcohol, water and ethanol). The peak of rutin from these solvents was identified by comparing their R_f obtained from the peak of standard. It was observed that the sample extracted using ethanol solvent showed maximum content of rutin. Further this solvent was used for extraction of rutin from plant samples collected from different geographical locations. Results are summarized in (table 4, figure 4)

Estimation of rutin from the whole plant extract of *M. pudica* collected from different geographical regions of India

The method was applied to evaluate the content of rutin from the whole plant of *M. pudica* collected from different geographical regions of India (Rajapur, Yeoor, Manglore, Guwhati and Nagothane). The peak of rutin from these samples was identified by comparing their R_f obtained from the peak of standard. It was observed that the sample collected from Manglore showed maximum content of rutin followed by Rajapur and least content of rutin was observed in the sample collected from Nagothane. Results are summarized in (table 5, figure 5). Further the samples collected from Manglore and Rajapur was subjected to different extraction techniques like vortexing, sonication and shaker (6hrs) since maximum content of rutin was estimated from these regions.

Estimation of rutin from the whole plant extract of *M. pudica* using different extraction techniques

The method was applied to evaluate the content of rutin from *M. pudica* collected from Manglore and Rajapur using different extraction techniques like sonication, overnight extraction and shaker since maximum content of rutin was estimated from these two regions. The peak of rutin from these samples was identified by comparing their R_f obtained from the peak of standard. It was observed that the sample collected from Manglore subjected to vortex technique showed maximum content of rutin. Results are summarized in (table 6, figure 6)

RESULTS AND DISCUSSION

Medicinal plants have made its niche in traditional system of medicines since ancient era (Ganeshpurkar and Saluja, 2017). Collection of these medicinal plants at proper time is very important in terms of their bioactive marker as the variation in these phytomarkers can impair its therapeutic efficacy. The therapeutic activity is due to the presence of several classes of secondary biologically active metabolites like alkaloid, glycoside, flavonoid and tannins, resins, glycosides etc. On preliminary screening of these secondary metabolites, flavonoids, tannins, alkaloids, glycosides, resins were found to be present as per their chemical tests (Khandelwal, 2008). Proximate parameters such as foreign organic matter, loss on drying, ash values (total, acid insoluble and water soluble) and extractive values (ethanol soluble and water soluble) of *Mimosa pudica* whole plant were determined (Table 1). The water soluble extractive value was found to be maximum. This suggests the presence of more polar components in the *Mimosa pudica*. Thus Standardization of medicinal plants is essential in order to assess its quality, based on the concentration of their active biomarkers (Shailajan et al., 2017 and Kamboj, 2000). HPTLC a quality control tool was used as a standardization parameter, method was developed and validated as per ICH guidelines to quantitate the content of rutin from plant samples collected from various geographical regions of India, extraction techniques and solvents of varying polarity. The LOD and LOQ levels were found to be 5µg/mL and 10µg/mL, respectively with a linear response range of 10-125 µg/mL and a correlation coefficient (r^2) value greater than 0.99. The content of rutin was quantitated using ethyl acetate: methanol: formic acid: water (10:2:1:1, v/v/v/v) as mobile phase. The R_f of rutin was found to be 0.47 under optimized chromatographic conditions. The content was found to be found to be 0.52899±0.00056 mg/g in the sample collected from Manglore, extracted 0.61201±0.00523 mg/g using ethanol as extracting solvent and 0.62873±0.00066 mg/g using vortex mixed as extracting technique.

CONCLUSION

The developed validated method is simple, precise, accurate and sensitive and can be used as quality-control check for plant extracts or poly-herbal combination containing *M. pudica* which will aid in standardization and prevent its adulteration, as there are many species of *M. pudica* which are deliberately or in-deliberately substituted with the other low quality and morphologically similar medicinal plants which tend to reduce the therapeutic efficacy (Shailajan et al., 2016). These methods can be applied to other plant raw materials containing the same phytochemical marker. The exact time and methods of harvesting, drying, storage and processing have an effect not only on morphological part but also in content of phytochemicals. This method can be used as quality control tool for the quality evaluation of *M. pudica* and formulation containing *M. pudica* as one of the ingredient.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST- NIL.

Table 1: Results of physicochemical parameters of *M. pudica* (Whole plant)

Parameters		Results
	Foreign organic matter	0.3361±0.0364
	Loss on drying	10.8499±0.7166
Ash content	Total	5.7892±0.1841
	Acid insoluble	2.2149±0.4132
	Water soluble	0.2184±0.0378
Extractive value	Ethanol soluble	14.0733±0.4962
	Water soluble	18.5332±2.5092
Values are (% Mean± S.D., n=3); (Ayurvedic Pharmacopoeia of India; Jadhav, 2012 and Nair, 2007)		

Table 2: Results of Phytochemicals in *M. pudica* (Whole plant) detected as per preliminary test

Phytochemical Constituents	Tests	Observation	Inference
Flavonoids	Ethanolic extract +increasing amount of NaOH	Yellow precipitate was observed	+
	Ethanolic extract + Lead acetate	Yellow precipitate was observed	+
Tannins	Aqueous extract + 5% $FeCl_3$	No deep blue colour was observed	-
	Aqueous extract + $K_2Cr_2O_7$	Red precipitate was observed	+
	Aqueous extract + Lead acetate	White precipitate was observed	+
Alkaloids	Ethanolic extract + Wagner's reagent	Orange brown precipitate was observed	+
Glycosides	Ethanolic extract + 1.0 mL H_2O + NaOH	Yellow coloration	+
Essential Oils	Ethanolic extract + drops of Vanillin Sulphuric acid	No white crystals	-
Resins	Boiled aqueous extract + conc. H_2SO_4	Reddish brown colour was observed	+
(+) :present; (-) :absent (Khandelwal, 2008)			

Table 3: Results of method validation experiment for estimation of Rutin using HPTLC

Parameters	Results for Rutin
Mobile phase	Ethylacetate: Methanol: Formicacid: Water (10:2:1:1v/v/v/v)
R_f	0.47
LOD and LOQ (µg/mL)	5 and 10
Linearity (µg/mL)	10-125
Regression equation	y = 69.219x - 72.343
Coefficient of determination (r^2)	0.995
Intraday Precision (% RSD)	1.05
Interday Precision (% RSD)	1.08
Recovery (%)	87%
Specificity Ruggedness	Specific, rugged
***Mean±SD, n=3	

Table 4: Results for estimation of rutin from the whole plant of *M. pudica* using solvents of varying polarity

Samples in different solvents	Content of rutin (mg/g)*
Hydro alcohol (7:3 v/v)	0.54474±0.00637
Ethanol	0.61201±0.00523
Methanol	0.23257±0.00210
Ethyl acetate, toluene, water, hexane	NA
***Mean±SD, n=3	

Table 5: Results for estimation of rutin from *M. pudica* ethanolic extract collected from different geographical regions of India

Sample from different geographical regions of India	Content of rutin (mg/g)*
Rajapur	0.51403±0.00582
Manglore	0.52899±0.00056
Guwhati	0.08861±0.00108
Nagothane	0.05358±0.00582
Yeoor	NA
*Mean±SD, n=3	

Table 6: Result for estimation of rutin from the whole plant extract of *M. pudica* using different extraction techniques

Sample using different extraction techniques	Content of rutin (mg/g)*
Rajapur	
Sonication	0.16947±0.00041
Shaker	0.51602±0.00583
Manglore	
Vortex	0.62873±0.00066
Sonication	0.51358±0.00052
Shaker	0.53779±0.00571
*Mean±SD, n=3	

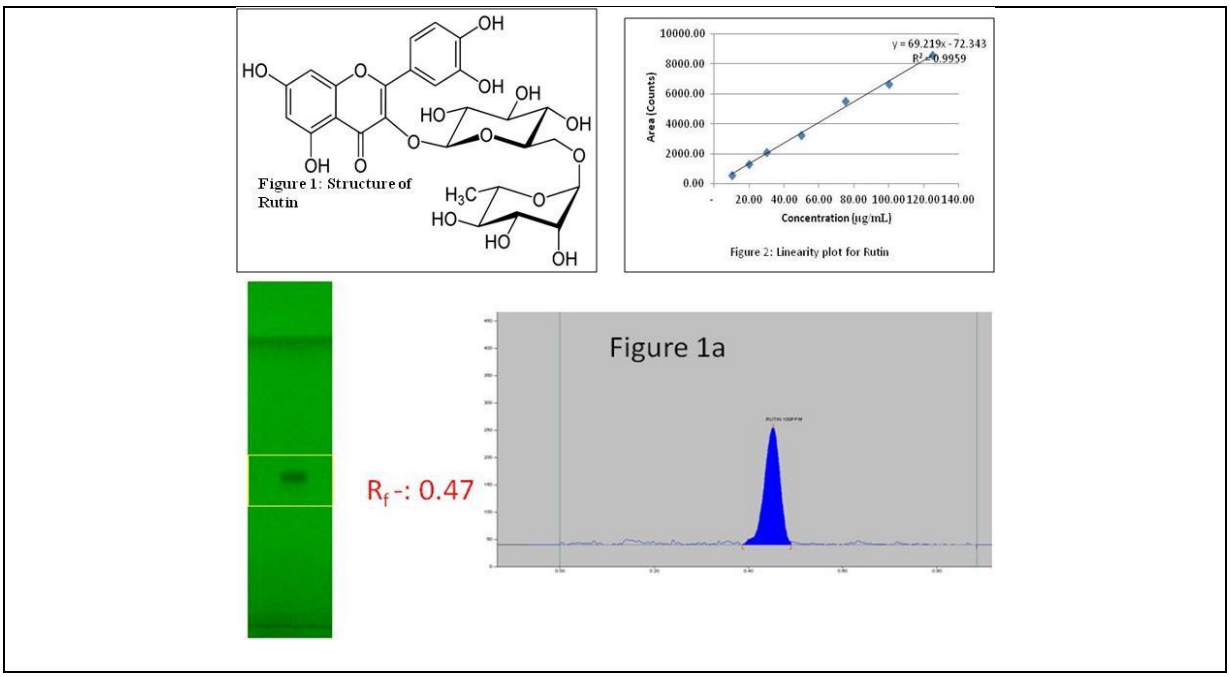


Fig. 1, 1a, 2: Estimation of Rutin

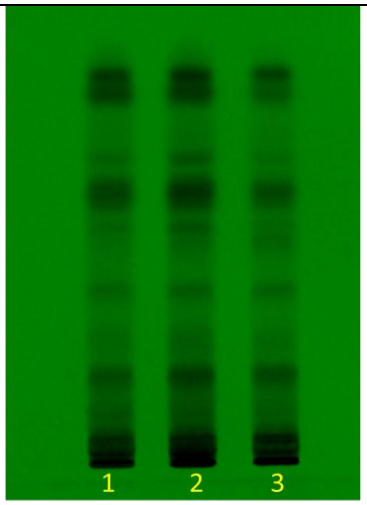
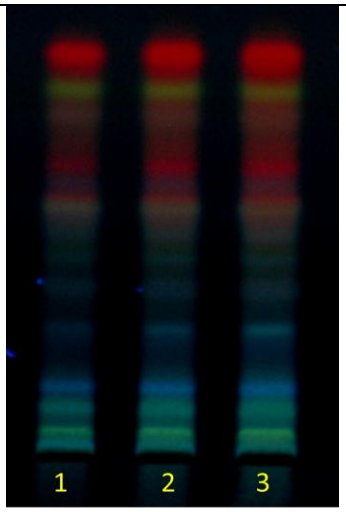
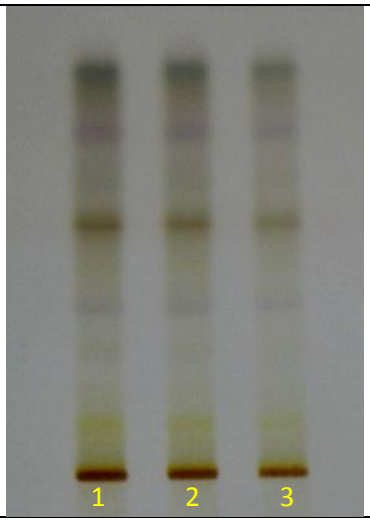
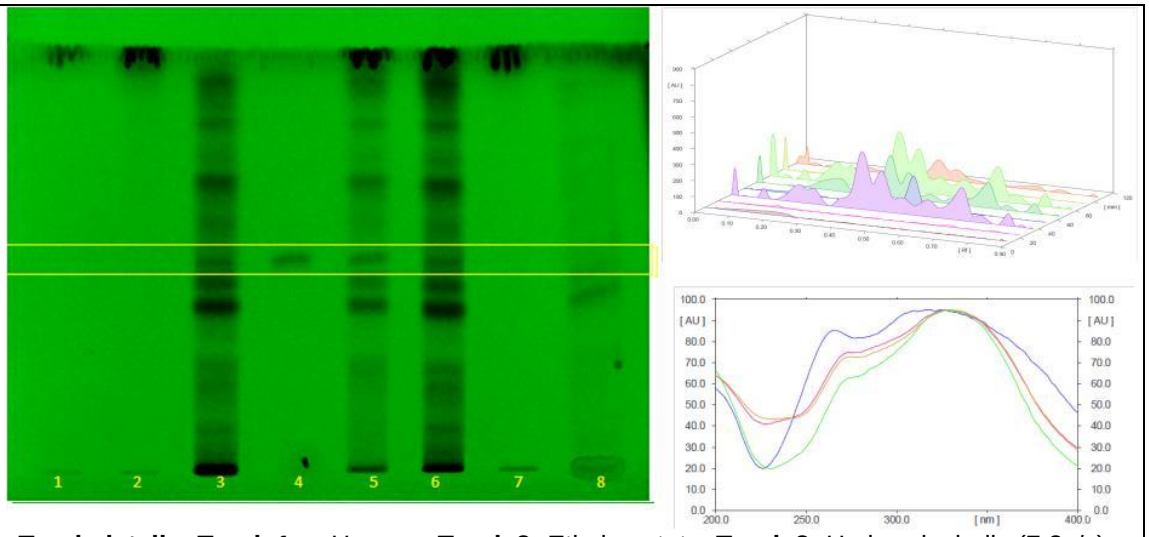
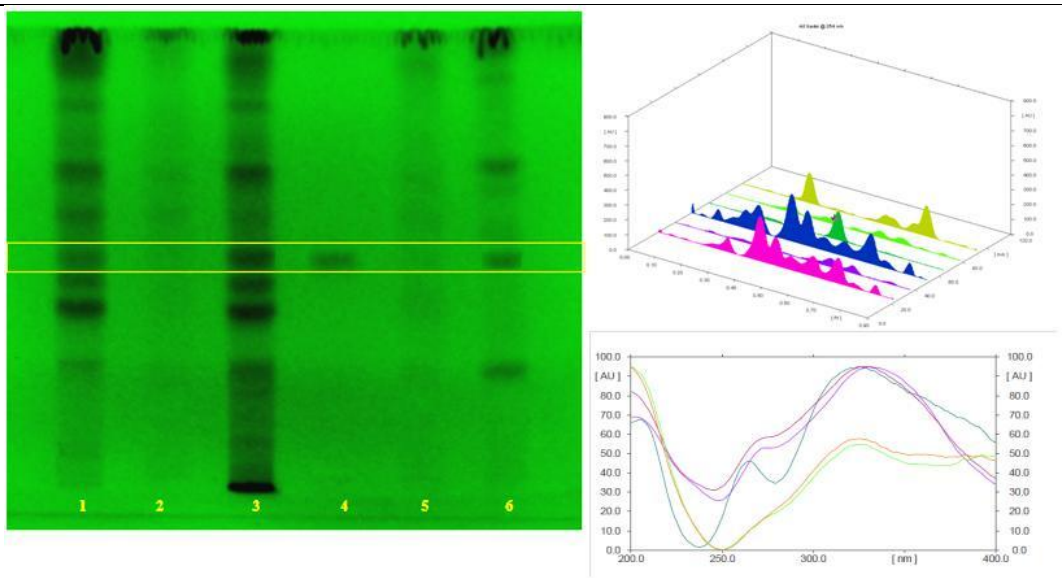
Before derivatization	After derivatization	
254nm	366nm	550nm
		

Fig. 3: Phytochemical fingerprint of *Mimosa pudica* (Whole plant)



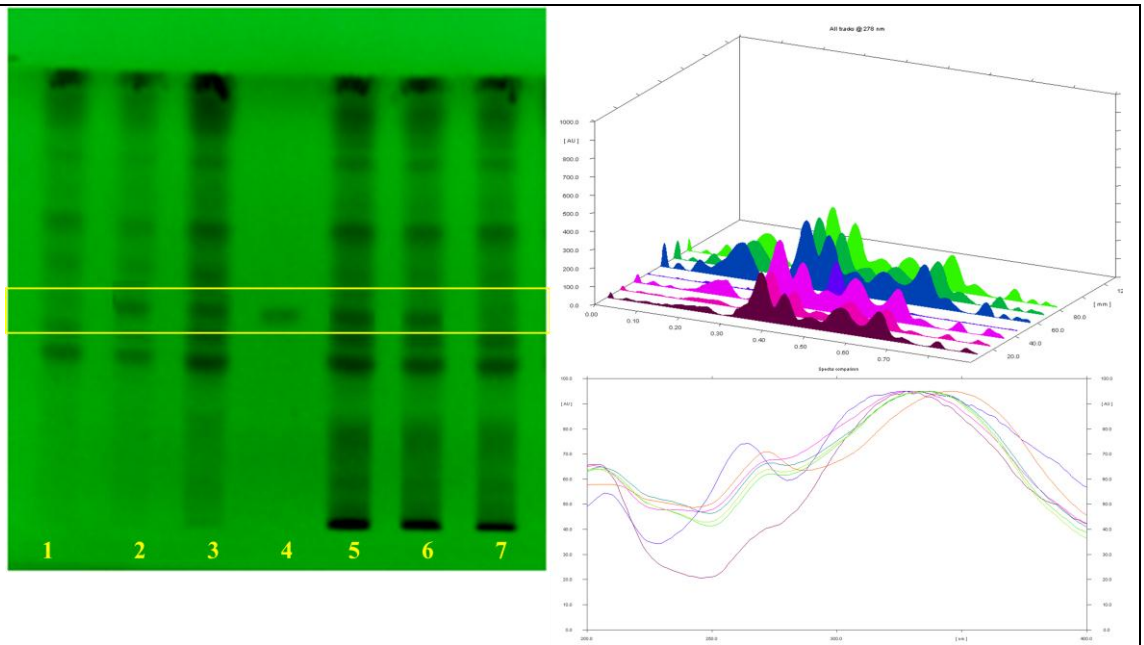
Track details: Track 1: n-Hexane, **Track 2:** Ethyl acetate, **Track 3:** Hydro-alcoholic (7:3v/v), **Track 4:** Rutin (100 ppm), **Track 5:** Ethanol, **Track 6:** Methanol, **Track 7:** Toluene, **Track 8:** Water.

Fig. 4: Plate photo for estimation of rutin from *M. pudica* (whole plant) using solvents of varying polarity at 254nm



Track details: Track 1: Rajapur, **Track 2:** Yeoor, **Track 3:** Manglore, **Track 4:** Rutin (100 ppm), **Track 5:** Guwhati, **Track 6:** Nagothane.

Fig. 5: Estimation of rutin from *M. pudica* (whole plant) collected from different geographical regions of India at 254nm



Track details: Track 1: Rajapur (Vortex), **Track 2:** Rajapur (Sonication), **Track3:** Rajapur (Shaker), **Track 4:** Rutin (100 ppm), **Track 5:** Manglore (vortex), **Track 6:** Manglore (Sonication), Track 7: Manglore (Shaker).

Fig. 6: Estimation of rutin from *M. pudica* (whole plant) using different extraction techniques

REFERENCES

1. Ahmed MF and Rao AS. Estimation of rutin in ethanolic extract of Brassica oleracea L. var capitata leaves by HPTLC method. International Journal of Scientific Research. 2013;2(8):421-422.
2. Anonymous. Indian pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad. 2010;1:82-201.
3. Azmi L, Singh MK and Akhtar AK. Pharmacological and biological overview on Mimosa pudica Linn. International journal of pharmacy & life sciences. 2011;2(11):1226-1234.
4. Choudhary N and Sekhon BS. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res. 2011;2(2):55-70.
5. Dharmendra S, Surendra JK, Sujata M and Shweta M. Natural Excipients- A Review. International Journal of Pharmaceutical & Biological Archives. 2012;3(5):1028-1034.
6. Ganeshpurkar A and Saluja A. Pharmacological potential of Rutin. Saudi Pharmaceutical Journal. 2017;25:149-164.
7. Jadhav M. Evaluation of Polyherbal combination of Amorphophallus campanulatus, Mimosa pudica and Symplocos racemosa in the management of chemically induced polycystic ovary in the rat model: A Bioanalytical Approach. (Thesis). 2012.
8. Joseph B, George J and Mohan J. Pharmacology and traditional uses of Mimosa pudica. International Journal of Pharmaceutical Sciences and Drug Research. 2013;5(2):41-44.
9. Kamboj VP. Herbal medicine. Current science. 2000;78(1).
10. Kandelwal KR. Practical Pharmacognosy Techniques and Experiments, Nirali Prakashan Publishers. 2008.
11. Naif AA, Mariadhas VA, Chang HP and Sang UP. An up-to-date review of rutin and its biological and pharmacological activities. EXCLI Journal. 2015;14:59-63.
12. Nair L. Standardisation of Medicinal Plants for the treatment of Reproductive disorders using the Female Rat. (Thesis). 2007.
13. Anonymous. Quality standards of Indian medicinal plants. Vol-9, Indian Council of Medical Research, New Delhi. 2011;211-219.
14. Shaikh Z, Roy SP, Patel P and Gohil K. Medicinal value of Mimosa pudica as an anxiolytic and antidepressant: a comprehensive review. World journal of pharmacy and pharmaceutical sciences. 2016; 5(3):420-432.
15. Shailajan S, Menon S, Singh D, Swar G and Bhosale S. Application of modern RP-HPLC technique for the quantitation of betulinic acid from traditional drug Symplocos racemosa Roxb. Journal of Applied Pharmaceutical Science. 2017;7(03):129-134.
16. Subramanian G, Meyyanathan SN, Byran G, Karthik Y and Palanisamy DS. Development and validation of liquid chromatography-mass Spectrometric method for simultaneous estimation of quercetin and rutin from Aganosma dichotoma [roth] k. Schum. Indo American Journal of Pharmaceutical Research. 2015;5(11):3412-3417.
17. Tamilarasi T and Ananthi T. Phytochemical analysis and anti-microbial activity of Mimosa pudica Linn. Research Journal of Chemical Science. 2012;2(2):72-74.
18. The Ayurvedic Pharmacopoeia of India (API). Part-I, Vol-II, First ed. Controller of Publications Civil Lines. New Delhi. 1999;98-101.