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RESEARCH ARTICLE

ESTIMATION OF P-COUMARIC ACID IN AQUEOUS EXTRACT OF *MIMSOA PUDICA* USING HPLC-UV METHOD

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ABSTRACT

A detection technique was developed for the analysis of p-coumaric acid which is found to be in ester-linked form in *Mimosa pudica* (MP). Aqueous extract of MP was prepared by using Soxhlet apparatus and thereafter quantification was performed by HPLC with a UV-VIS detector. A reversed-phase C18 column was used as stationary phase and the optimal condition was established with (Water (50µl of formic acid pH adjusted to 3): acetonitrile (90:10)). To reduce the time of sample processing during quantification the extraction of ester-linked phenolic acid was performed using Soxhlet extraction technique our technique lead to rapid detection of p-coumaric acid using HPLC-UV. On quantification leaves of *Mimosa pudica* were found to contain 3.35% w/w of p-coumaric acid.

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INTRODUCTION

p-Coumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid) is a ubiquitous plant metabolite with antioxidant, anti-inflammatory, and anticancer properties (Kadoma and Fujisawa, 2008 and Rocha *et al*., 2012). This acid is present in plants in both free and bound forms where a small fraction occurs as "free acids" and the majority are linked to structural components of the plant (Khandelwal, 2008). The majority of p- coumaric acid is esterified to lignin (Rocha *et al*., 2012). The objective of this work was to propose an alternative method that is fast and precise. This paper describes the development of a new method using high performance liquid chromatography (HPLC-UV) for the separation of p-coumaric acid in aqueous extract (MPAQ) of *Mimosa pudica.*

MATERIALS AND METHODS

Materials

Acetonitrile (HPLC grade) water (HPLC grade), ethanol (LR), methanol (LR), p-coumaric acid (EP) were purchased from Merck and Sigma alderich.

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Plant material

The plant *Mimosa pudica* (MP) was collected from garden at Badlapur and 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 water as solvent to get aqueous extract (MPAQ) (Anees *et al*., 2009). Extract was air dried. The dry extract was stored in an air- tight container in refrigerator (5^0 +/- 1^0 c) for experimental use (Harbone, 1998 and Anees *et al*., 2009).

Preparation of standard and sample solutions

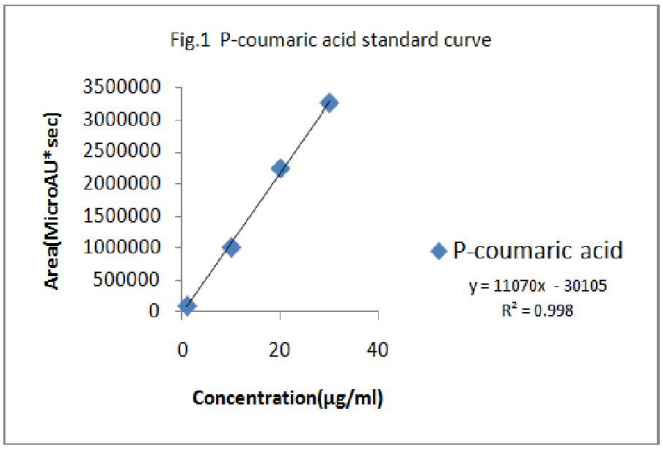
P-coumaric acid (10 mg) was dissolved in 10 ml of methanol (10 ml) to yield solution of 1000 µg/ml and this solution was further diluted equimolarly with methanol to yield solution of 10 µg/ml. MPAQ extract (40 mg) was dissolved in 10 ml of HPLC grade water to yield solution of 4000 µg/ml and was used for quantification purpose under following chromatographic conditions.

Chromatographic conditions- HPLC

HPLC System-	Jasco HPLC system
Pump-	Jasco PU-2089 Plus Quaternary Gradient HPLC Pump
Detector-	Jasco MD-2018 Plus PDA Multiwavelength Detector
Integrator-	Chromnav software
Stationary phase-	BDS C18
Mobile phase-	Water(50µl of formic acid pH adjusted to 3) : acetonitrile (90:10)
Detection wavelength-	315 nm
Flow rate-	1ml/min
Sample size-	20 µL

p-coumaric acid was quantified using HPLC method. Standard and extract solutions were prepared and injected into column (injection volume was 20µl). BDS C18 column used as stationary phase while, water (50µl of formic acidused to adjust pH adjusted to 3) acetonitrile & water (90:10) used as mobile phase. Flow rate was maintained at 1ml/min.

Detection wavelength was 315 nm which was λ_{max} of p-coumaric acid. Chromnav software was used for calculations (Harbone, 1998; Santos *et al*., 2011 and Khandelwal, 2008).



HPLC chromatograms

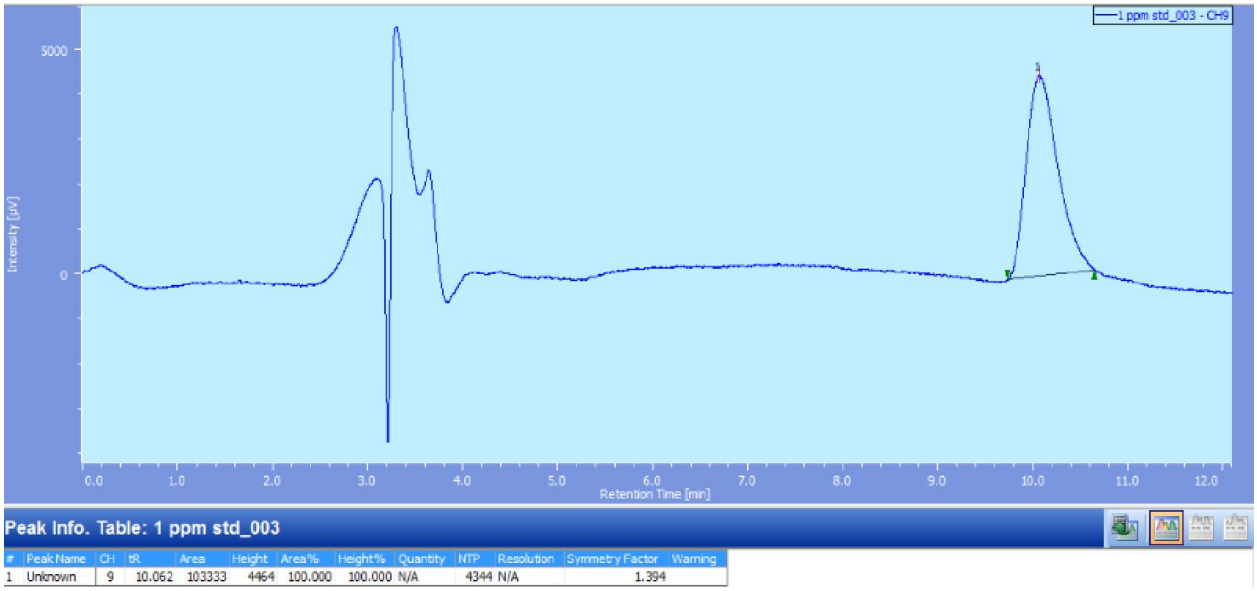


Fig.2. Standard (1 ppm)

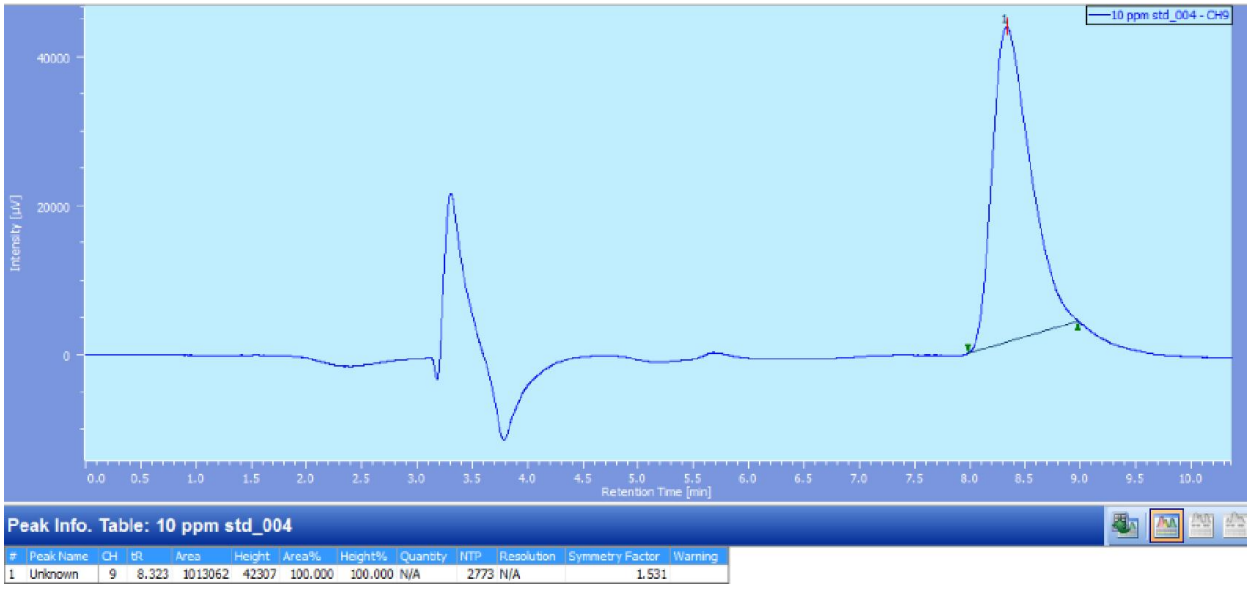


Fig. 3. Standard (10 ppm)

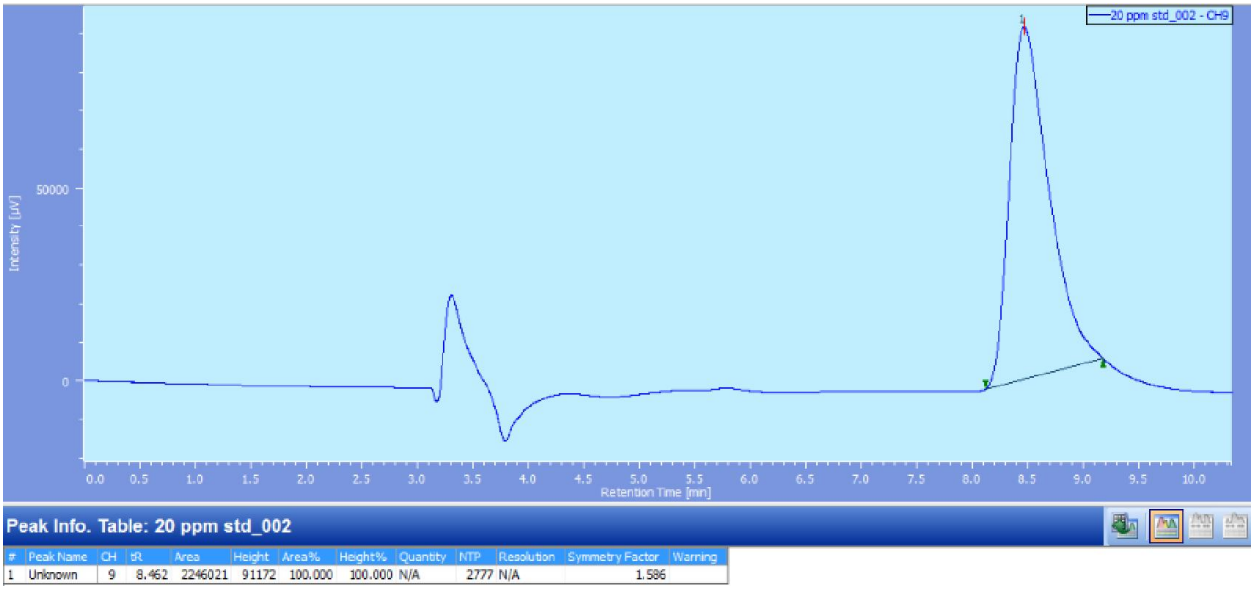


Fig.4. Standard (20 ppm)

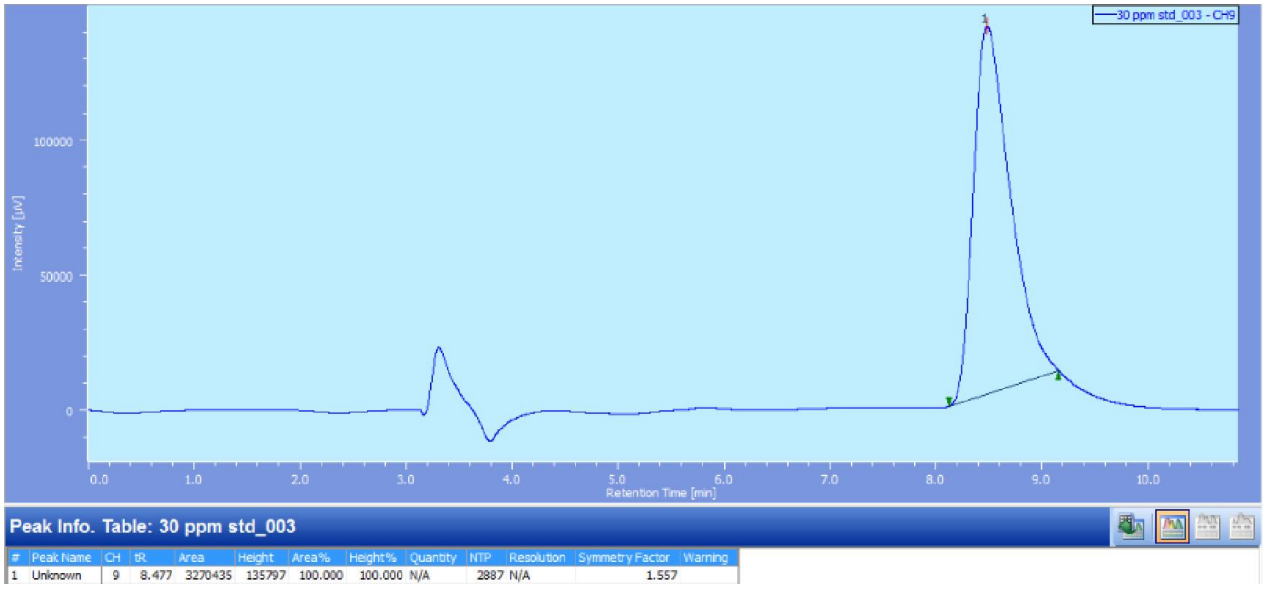


Fig.5. Standard (30ppm)

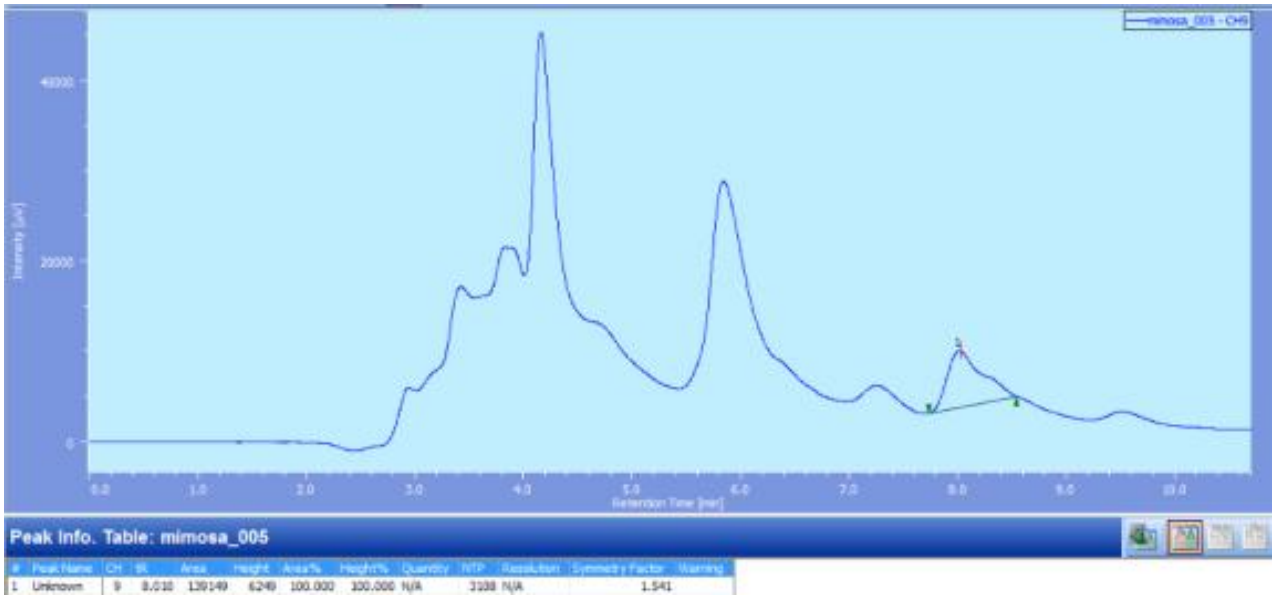


Fig.6. MPAQ extract

RESULTS

Sample	Concentration	Peak area	Concentration of p-coumaric acid in MPAQ extract
Std(p-coumaric acid)	1 µg	103333	-
Std(p-coumaric acid)	10 µg	1013062	-
Std(p-coumaric acid)	20 µg	2246021	-
Std (p-coumaric acid)	30 µg	3270435	-
MPAQ extract	4000 µg	139149	1.34 mg

DISCUSSION

In *Mimosa pudica* phenolic acid found to be present is p-coumaric acid. It is found to be bound to the lignin with ester linkage. Bound form of p-coumaric acid leads to difficulty in estimation of amount present in the plant. Hence there is a need to develop the method which can preciously detect amount of p-coumaric acid present in leaves of *Mimosa pudica*. In our study extraction was performed using Soxhlet apparatus instead of conventional methods (maceration, ultrasonic extraction) which lead to maximum pulling out of p-coumraic acid from MP such extract rich with phenolic acid and set free p-coumaric acid.

This unbound p-coumaric acid was found to be detected by HPLC rapidly with less than 10 min when run through BDS C18 column. Mobile phase used was water (50µl of formic acid pH adjusted to 3): acetonitrile (90:10) which helps to separate the p-coumaric acid with the retention time less than 10 min. This method thus provide rapid detection technique for p-coumaric acid present in leaves of *Mimosa pudica* using HPLC-UV.

Conclusion

This study has demonstrated the potential use of HPLC-UV method for detection of phenolic acid such as p-coumaric acid in *Mimosa pudica* extract. The further research can be done to develop the method for detection of other phenolic acids present in leaves of *Mimosa pudica.* possible modify the extraction methods and determination of the phenolic compounds in the *Mimosa pudica* extract. Extraction technique can also be modified to facilitate precise isolation of p-coumaric acid with greater yield.

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