ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Standardization of *Coscinium fenestratum* with reference to berberine by high performance thin layer chromatography.

Krishnan Chitra, Kuppusamy Sujatha*, Haritha Polisetti, Sarika Karri, C Umamaheswara Reddy.

Dept .Pharma Chem, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai-600116, Tamil Nadu, India.

ABSTRACT

A simple and reproducible HPTLC method for the determination of berberine in *Coscinium fenestratum* was developed and described. The HPTLC method involves separation of components by TLC on precoated silica gel 60 F 254 plate with a solvent system of cyclohexane : chloroform : glacial acetic acid (4.5 : 4.5 : 1) and detection at 366 nm in absorbance mode. The sensitivity of HPTLC method was found to be 0.20µg and the linearity was observed in the range of 0.2 µg to 5.00 µg.The berberine content of 4.67% was observed in the test sample.

Keywords: Coscinium fenestratum, HPTLC, berberine, standardization.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Coscinium fenestratum (Gaertn), Menispermaceae is one such plant which is widely used in indigeneous system of medicine. The stem portion of the plant is suggested to have thermogenic, anti-inflammatory, antiseptic and tonic effects and is used against opthalmopathy, inflammations, ulcers, jaundice and general debility [1, 3, 4]. Literature survey revealed hepatotoxicity effects [7], antioxidant activity [6], hypotensive activity [5] and antinociceptive effects [2] of stem extracts of this plant. In the present work, a suitable, sensitive and reliable quantitative High Performance Thin Layer Chromatographic method has been developed for the quality control determination of berberine from *C.fenestratum.*

MATERIALS AND METHODS

The woody climbing stem of *C.fenestratum* was collected from Mannarghat area of Palghat district, Kerala, India during April-May 2009 and identified in our Pharmacognosy department, where a voucher specimen is maintained. The stem part of the plant was chopped, air dried at 35-40 ◌̊C for 2 months and pulverized in an electric grinder. Chloroform extracts were prepared from the stem part and the yield was found to be 10 %w/w. 2 and 5 µl of methanol extract were applied as bands of silica gel 60 F 254 precoated aluminium plates (Merck) along with authentic sample of standard berberine (5 µl) using linomat IV sample applicator. The speed of application was maintained at 10 µl/sec.The width of the band was kept at 6 mm. The chromatogram was developed up to 80 mm under chamber saturation condition, using the mobile phase cyclohexane: chloroform: glacial acetic acid (4.5:4.5:1). The plate was air dried and scanned at 366 nm in absorbance mode. The amount of berberine was determined using the calibration curve plotted between concentrations and area of standard berberine. (0.10 mg/ml in chloroform). The equation for berberine was found to be y=10873 x +778.9 with a correlation coefficient of 0.998 where y is the response in peak area and x is the concentration in mg/ml. The contents of berberine were quantified using proposed method and % recovery calculated.

Method Validation and Recovery

A varying known amount of berberine was added to 1 g of the chloroform extract in which the contents of berberine had been estimated previously by proposed method. The sample were extracted and analyzed separately as per the procedure mentioned above. The results were provided in table 1.

ISSN: 0975-8585

Table 1: Recovery of berberine from *C.fenestratum*

S.no	Sample	Amount of Extract taken (mg)	Amount of Berberine in A (mg)	Amount of Berberine Added to A (mg)	Amount of Berberine Taken B +C (mg)	Total Berberine present	% Recovery E/Dx100
		A	B	C	D	E	
1	Chloroform ExtractOf C.fenestratum	1020	48.62	2.00	50.62	50.55	99.86
2	Chloroform Extracto f C.fenestratum	1010	48.52	2.00	50.52	50.18	99.32
3	Chloroform Extract of C.fenestratum	1008	48.28	2.00	50.28	50.26	99.96

RESULTS AND DISCUSSION

By trying different composition of mobile phase, the desired resolution of berberine with symmetrical and reproducible peaks are achieved by using cyclohexane: chloroform: glacial acetic acid (4.5 : 4.5 : 1). The Rf value of berberine was found to be 0.75. The HPTLC chromatograms of standard berberine and test sample are shown in fig 1 and 2. The % recovery of berberine was found to be 99.32 to 99.96%.

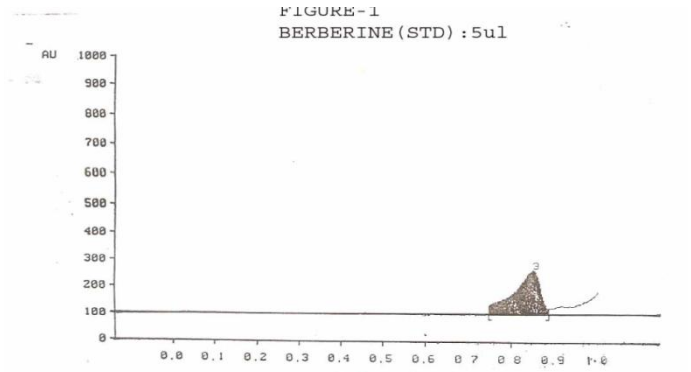
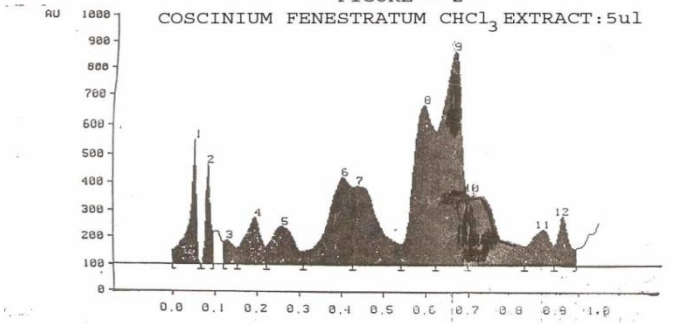


FIGURE - 2
COSCINIUM FENESTRATUM $CHCl_3$ EXTRACT:5ul



ISSN: 0975-8585

The calibration curve was linear in the range of 0.2 to 5 µg for berberine. Further a correlation coefficient 0.998 indicates good linearity between concentration and area.

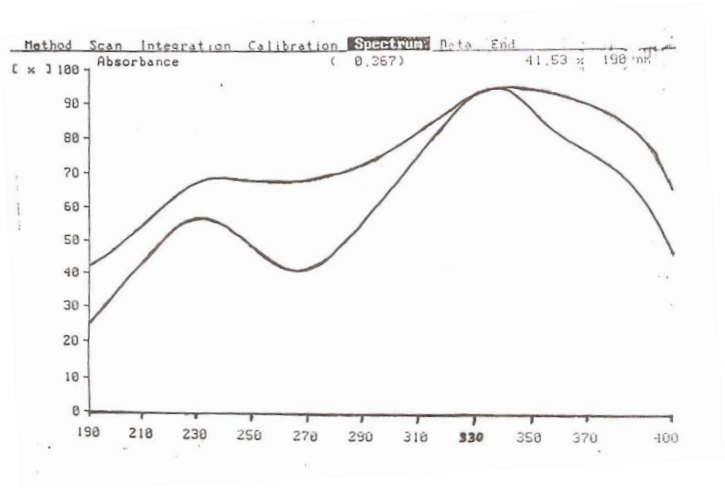


Fig. 3: SCAN INTEGRATION CALIBRATION SPECTRUM DATA END

To ascertain the purity of peak in test sample, its insitu reflectance spectrum was compared with standard berberine which provides clear super impossibility indicating the purity of peaks (fig-3). Further recovery values of 99.32 to 99.96 (99.92 ± 1.52 %) were obtained showing the excellent reliability and reproducibility of proposed method. The proposed HPTLC method is rapid, simple, sensitive, precise and accurate for quantitative monitoring of berberine in *C.fenestratum* and can be used for routine quality testing.

REFERENCES

[1] Cains JE. The Medicinal and poisonous plants of India (1st edn), scientific publishers:Jhodhpur, India.1986; 171.
[2] Chitra K, sujatha K, Dhanuskha SH, Mangathayaru K, Vasantha J, Janani S, Janani K. Biomedical research 2004; 15(1): 73-75.
[3] Kiritikar KR, Basu BD. Indian medicinal plants Orient Longman; Chennai. 1933; 2nd Ed. Vol 1: 84.
[4] Nadkarani AK. Indian Meteria Medica. Popular Prakashan; Mumbai. 1954; 3rd Ed. Vol II: 318.
[5] Singh GB, Singh S, Bani S, Malhotra S. J Ethnopharmacol 1990; 30: 151-155.
[6] Venu kumar MR, Latha MS. Ind J Physiol pharmacol 2002; 46: 223-228.
[7] Venu kumar MR, Latha MS. Ind J Exp Biol 2004; 42: 792-797.