

## HPTLC ANALYSIS OF BERBERINE FROM *TINOSPORA CORDIFOLIA* STEM EXTRACT

Shivam Singh<sup>1\*</sup>, Pragma Singh<sup>1</sup>, Abhinai Singh<sup>1</sup>, Arvind Tripathi<sup>1</sup>, Shrikant Kaul<sup>1</sup>, Riya Shrivastava<sup>2</sup>,  
R. M. Mishra<sup>2</sup>

<sup>1</sup>Centre for Biotechnology and Microbiology Studies, A.P.S University, Rewa, (M.P.), India- 486003

<sup>2</sup>Department of Environmental Biology, A.P.S University, Rewa (M.P.), India- 486003.

\*Author for Correspondence: Shivam Singh

Centre for Biotechnology and Microbiology Studies, A.P.S University, Rewa, (M.P.), India- 486003.

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### ABSTRACT

Estimation of Berberine from High-performance thin layer chromatographic method was developed for ancient Indian medicinal climber *Tinospora cordifolia* based on its specificity and accuracy. The shed dried stem of *Tinospora cordifolia* was subjected to Methanolic extraction with a good yield of 4.98 (W/W). A TLC aluminium sheet precoated with silica gel 60 F254 was used as the stationary phase. The mobile phase system consisting of Toluene: Ethyl acetate at ratio 9:3 (v/v) with Rf value of 0.05 produces a good resolution of Berberine. Scanning analysis was performed at an absorbance wavelength of 266 nm. The calibration graph of Berberine was obtained in the range of 1.0 to 7.0 µL/spot and exhibit appropriate correlation coefficient 0.9994. The intra-day and inter-day precision analysis of Berberine was found to be 0.32 and 0.38, respectively. The concentration of Berberine in the stem of *Tinospora cordifolia* with respect to dry weight basis was found to be 0.945 mg (w/w) with Mean % RSD of 1.41. Statistical investigations proved that the proposed process is precise and reproducible. The anticipated method was also helpful for the assessment of Berberine from crude medicinal formulations containing *Tinospora cordifolia*.

**KEYWORDS:** *Tinospora cordifolia*, Berberine, Methanolic.

### INTRODUCTION

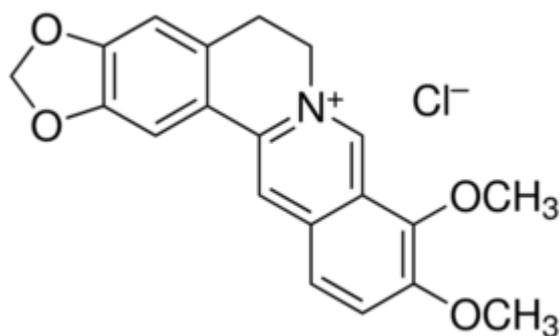
All over the world scientists investigate plants, micro-organisms and many other forms of life for biologically active compounds. Research is directed towards interactions between organisms that can be attributed to a chemical substance present in at least one of the species concerned. Of greatest interest is the effect of extracts from tribal plants on human physiology and human pathology, since this is very relevant to the discovery of new drugs for treating diseases of human beings and other mammals. Most of the earliest pharmaceuticals were plant materials. A number of investigations have evolved into a search for new biochemical targets, the development of bio-assays, and high throughput screening of as many compounds as possible to find chemical structures for drug development.<sup>[1]</sup> The effect on human health and activity following the ingestion or application of plant products is known in most societies and the use of plants for treating diseases started before written history.

*Tinospora cordifolia* which commonly known as Guduchi has a long history of use in traditional medicine, has recently been shown to have efficacy in the treatment

of diabetes.<sup>[2]</sup> and has been used in Ayurvedic preparations for the treatment of various disorders throughout the centuries. It is used as an ancient medicine to improve the immune system and body resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India. The alcoholic and aqueous extracts of *Tinospora cordifolia* are reported to have beneficial effects on liver damage either by Kuffer cells stimulation or by other means.<sup>[3,4,5]</sup> and have also been tested successfully for their immune modulatory activity<sup>[6,7]</sup> as well as anti arthritis activity.<sup>[8,9]</sup>

These medicinal values are often due to higher alkaloid content out of which recently identified amritosides A, B, C and D (clerodane furano diterpene glucosides) have been isolated as their acetates from stems.<sup>[10]</sup> Other components such as diterpenoid lactones, glycosides, polysaccharides, steroids, sesquiterpenoid, phenolics, aliphatic and aromatic compounds. Leaves of this plant contain protein (11.2%), calcium and phosphorus.<sup>[11]</sup> One of such well known bioactive component is Berberine (Figure 1) which is found in large amount in *T. cordifolia*

stem extract.<sup>[12]</sup>



**Figure 1: Chemical structure of Berberine.**

Berberine has been shown activity against fungal infections, *Candida albicans*, yeast, parasites along with bacterial and viral infections<sup>[13,14]</sup> Berberine seems to exert synergistic effects with fluconazole even in drug-resistant *C. albicans* infections.<sup>[15]</sup> Some research has been conducted in search for possible use against methicillin-resistant *Staphylococcus aureus* infections.<sup>[16]</sup> Berberine is considered as potent antibiotic.<sup>[17]</sup> when applied in vitro and in combination with methoxy hydnocarpin, an inhibitor of multidrug resistance pumps. Berberine inhibits growth of some gram positive bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*<sup>[18]</sup> and a toxic cyanobacterium *Microcystis aeruginosa*.<sup>[19, 20]</sup> Berberine is a useful medicine in the treatment of leishmaniasis.<sup>[21]</sup>

Out of several chromatographical techniques used for quantitative analysis HPTLC was selected due to its preciseness, accuracy and reproducibility along with its economical mobile phase consumption, as compared to HPLC.

#### MATERIAL AND METHOD

HPLC grade methanol, ethyl acetate, toluene, water and HPTLC silica gel plate 60F254 (20cm×20cm) etc are obtained from E.Merck chemicals (India). Biochemical standard Berberine as well as reverse phase HPLC columns sourced from Sigma-Aldrich chemicals (India). The purity of chemicals was checked by comparing the high purity (>99%) of HPLC standard.

A Camag - HPTLC system using tungsten lamp as radiation source comprising of CAMAG Linomat V automatic spotter with 100 µL Hamilton syringe, Camag TLC Scanner III with winCATS software, Camag UV-cabinet and Camag twin trough development Chamber (20x10cm) was engaged.

#### Preparation of standard Berberine solution

A Berberine solution of concentration 1µg/µl was prepared in methanol. Different amounts 1, 2, 3, 4, 5, 6

and 7 µl of standard was spotted in triplicate on silica gel 60F254 (20cm×20cm) TLC plate, using Camag Linomat V sampler (Camag, Switzerland) and a 100µl Hamilton syringe for preparing seven point calibration graph.

#### Preparation of crude Plant extract

*Tinospora cordifolia* whole plants are collected in the month of July from the Jayanti kunj, Rewa, (M.P.). Fresh and mature stem nodes of *Tinospora cordifolia* are collected and dried in the shade. Mature stem nodal parts are then coarsely powdered by mortar-pistol and then divided in to two equal parts. One part is oven dried at 60 °C for 72 hours after which the dried plant material was extracted with methanol, plant material filtered off and the methanol evaporated to dryness. The dried extract was further used to prepare a known concentration of the extract in methanol followed by filtration with 0.45µ filter paper. The filtered solution was applied on TLC plate followed by development and scanning.

#### Method used for Analysis

The silica gel 60F254 (20cm×20cm) TLC plate was dried and developed previously using mobile phase {Toluene: Ethyl Acetate (9:3, V/V)}. Twin trough chamber (20x10cm) was saturated and maintained at constant temperature 25 ± 5°C. Berberine standard solution of amount 1, 2, 3, 4, 5, 6 and 7 µl of concentration 1µg/µl along with previously processed sample were spotted in triplicate on TLC plate.

After development, plate was dried and analyzed at 266 nm wavelength. Calibration curve was produced by plotting respective peak areas of Berberine against concentration.

#### RESULTS AND DISCUSSION

The mobile phase system containing Toluene: Ethyl acetate at ratio 9:3 (v/v) gave a good resolution sharp peak of Berberine with R<sub>f</sub> value of 0.05 (Fig 2). The proposed method was validated as per ICH guidelines. Different Validation Parameters for proposed methodology was summarized in Table 1. Appropriate spot was obtained when the chamber was saturated with the mobile phase for 30 minutes. The calibration curve of Berberine was obtained in the range of 1.0 to 7.0 µL/spot with peak area (n=3) and exhibit proper correlation coefficient 0.9994 (Fig 3). The intra-day and inter-day precision analysis of Berberine was found to be 0.32 and 0.38, respectively. The concentration of Berberine in the stem of *Tinospora cordifolia* with respect to dry weight basis was found to be 0.945 mg (w/w) with Mean % RSD of 1.41. This HPTLC methodology is found to be simple, precise and accurate. The proposed method is sensitive for the analysis of Berberine in different medicinal formulations.

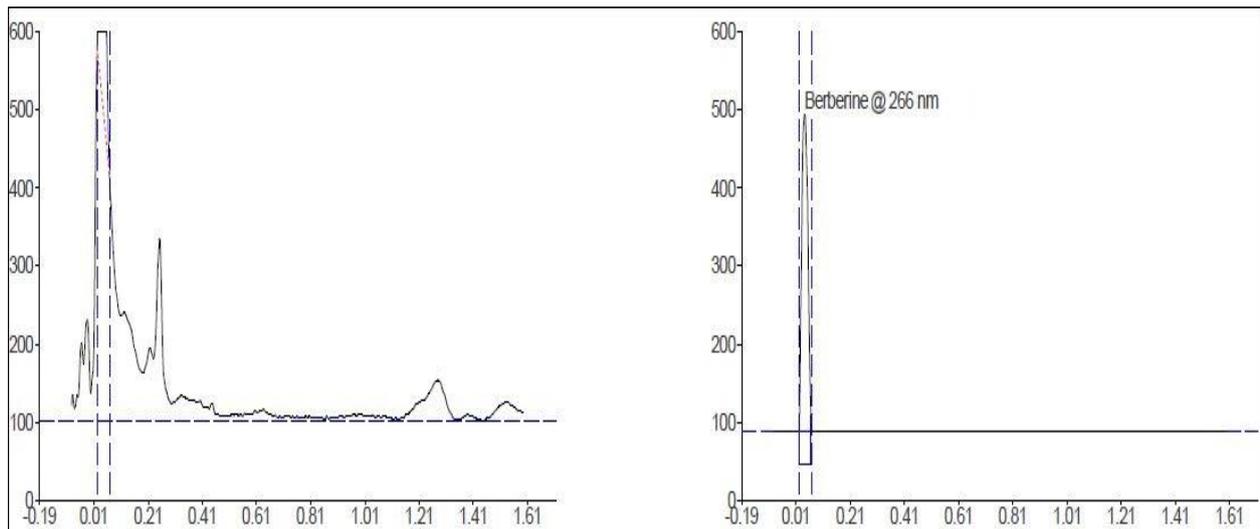


Figure 2: HPTLC chromatogram of Berberine in *Tinospora cordifolia* Extract (7µL/spot) with Rf 0.05.

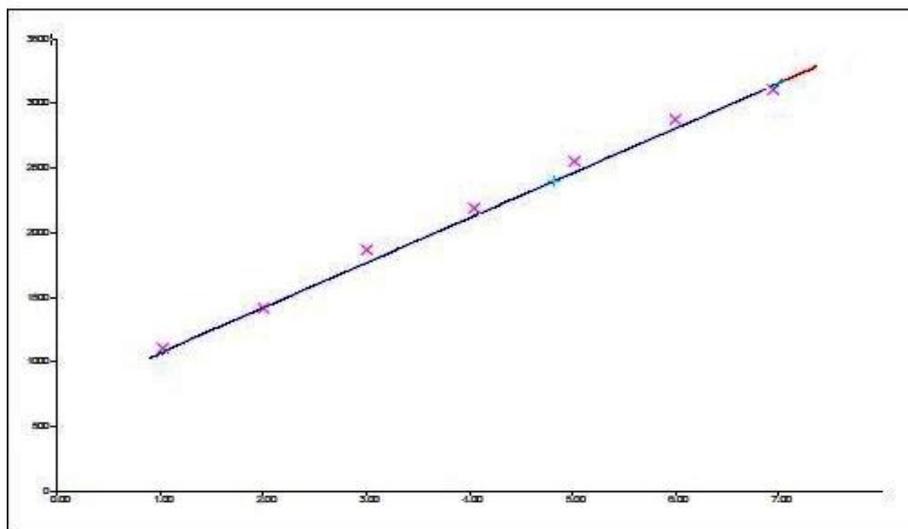


Figure 3: HPTLC calibration graph of Berberine for 1.0 to 7.0 µL/spot.

Table 1: Validation parameters for HPTLC proposed in the study.

Parameters Validated for Berberine Analysis	HPTLC
Linearity range	1-7 µg/spot
Correlation coefficient	0.9989
Limit of detection (LOD)	0.5 µg/spot
Limit of quantification (LOQ)	1 µg/spot
Stability	80 Minutes
Application repeatability (n=5)	0.42
Robustness	Present
Recovery (n=5)	98.4
Precision (R.S.D. %)	
Inter-day (n=5)	0.38
Intra-day (n=5)	0.32

## CONCLUSION

The proposed HPTLC method is simple, specific and cost-effective for the estimation of Berberine. The selected mobile phase and validation parameters appropriately resolve Berberine. The method is

successfully used for the estimation of Berberine in different pharmaceutical agents. This method can be used as a valuable analytical tool for screening and quantification as well as standardization of these formulations in laboratory and commercial purposes.

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