

Original Article

SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TOTAL ALKALOIDS IN THE *TINOSPORA CORDIFOLIA* M. AND ITS HERBAL FORMULATIONS

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ABSTRACT

Objective: A simple spectrophotometric method was developed for the estimation of total alkaloids in *Tinospora cordifolia* M. and its formulations.

Methods: The method based on the formation of yellow colored complex by reaction between bromocresol green (BCG) and alkaloids in medicinal plants. A yellow colored complex forms is easily extractable by chloroform at pH 4.7.

Results: The method was linear in concentration range of 2-10 µg/ml with λ_{max} at 415 nm. The LOD and LOQ were found to be 0.215 and 0.652 respectively. The intra-day and inter-day precision and accuracy were within the acceptable criteria (relative standard deviation < 2.0% and accuracy within 100±2%).

Conclusion: The developed method is simple, precise and accurate and can be adopted for the routine quality control and standardization of plant materials containing alkaloids and its pharmaceutical products.

Keywords: *Tinospora cordifolia* M, Total alkaloids, Bromocresol green (BCG).

INTRODUCTION

Stem of *Tinospora cordifolia* M. (Family: Menispermaceae), Known as Gaduchi in Sanskrit and Amrita or Giloya in Hindi, have long been used as antidiabetics, anti-inflammatory, antiarthrititis, antioxidant, antistress, hepatoprotective, antineoplastic. It contains various chemical constituents belongs different classes such as alkaloids, diterpenoids lactone, glycoside, steroids.

The therapeutic efficacy of *T. cordifolia* is due to presence of alkaloids of which Berberine chloride is major one [1-6]. From the literature review the titrimetry and spectrophotometry method for the estimation of total alkaloids in various plant samples and formulations have been reported, but none method was reported for estimation of total alkaloids in *T. cordifolia* M. [7-12]. In the present paper, a simple spectrophotometric method was developed for the estimation of total alkaloids in plant sample and its marketed formulations of *T. cordifolia*.

The stems powder of *Tinospora cordifolia* and its marketed formulations were purchased from local markets, Gujarat, India. Reference standard of Berberine chloride (HPLC Purity > 98%) was purchased from Sigma Aldrich, Karnataka, India. All other solvents were used of analytical grade.

Bromocresol green solution (BCG) (10⁻⁴M) was prepared by heating at 50-60 °C, 10-15 min of 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na₂HPO₄ in 1 l distilled water) to 4.7 (4.5 to 4.9) with 0.2 M citric acid (42.02 g citric acid in 1 l distilled water).

The method was performed on Shimadzu 1800 series double beam spectrophotometer with fixed slit width (1 nm) attached to computer with UV-probe version 2.33 software for obtaining the spectra.

Preparation of standard stock solution of Berberine chloride (100 µg/ml)

A standard solution of Berberine chloride (100 µg ml⁻¹) was prepared by dissolving 1.0 mg of accurately weighed Berberine chloride in 10 ml of volumetric flask using methanol.

Preparation of calibration curve of Berberine chloride (2-10 µg/ml)

Suitable aliquot (0.2, 0.4, 0.6, 0.8 and 1 ml) of the above standard stock solution was transferred to separating funnel. Add 5 ml of phosphate buffer pH 4.7 and 5 ml of bromocresol green (BCG) solution (10⁻⁴ M). The mixture was shaken and complex formed was extracted with 5 ml of chloroform. Chloroform layer was collected in 10 ml of volumetric flask and make the volume up to mark with chloroform. Absorbance was taken at 415 nm against the blank. The calibration curve was constructed by absorbance versus concentration of Berberine chloride as standard (µg/ml).

Estimation of total alkaloids in stem powder and formulations of *Tinospora cordifolia*

Each 2 g of the *Tinospora cordifolia* powder and its formulations were extracted separately with 20 ml of methanol three times at 50-60 °C. Collect and combined methanol extract and evaporated to dryness to get the residue. The residue was dissolved in 2 N of Hydrochloric acid (HCl) and then filtered. 1 ml above test solutions was transferred to separating funnel and add 5 ml of phosphate buffer pH 4.7 and 5 ml of bromocresol green (BCG) solution (10⁻⁴ M). The mixture was shaken and complex formed was extracted with 5 ml of chloroform.

Chloroform layer was collected in 10 ml of volumetric flask and make the volume up to mark with chloroform. Absorbance was taken at 415 nm against blank. The solutions were stable for 2 h. The total alkaloids were determined by the regression equation.

MATERIALS AND METHODS

Method validation

The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures [13-14].

Linearity

The linear response was determined by analyzing six independent levels of the calibration curve in the range of 2-10 µg/ml for Berberine chloride. The result should be expressed in terms of Correlation co-efficient.

Precision

Intra-day precision and Inter-day precision were determined for a standard solution of Berberine chloride (4, 6 and 8 µg/ml) for the three times on the same day for Intraday and on three different days for inter-day precision.

LOD and LOQ

Calibration curve was repeated 3 times and the standard deviation (SD) of the intercepts (response) was calculated. Then LOD and LOQ were measured by using mathematical expressions.

Accuracy (% Recovery)

Accuracy expressed as % Recovery by the assay of known, added amount of analyte. Its measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by previously analyzed test samples with three different concentrations of standards at 80%, 100% and 120% respectively.

The titrimetric and gravimetric method for estimation of total alkaloids in plant samples have a lack of sensitivity, in gravimetric method, the residue found to be impure since more than one spot revealed by TLC. While in tritrimetry method there was problem in end point detection due to interference of color of the extract. The proposed spectrophotometric method is based on the reaction between alkaloids and bromocresol green (BCG) to form yellow color complex, which can be extracted with chloroform at pH 4.7 and measured at λ_{max} of 415 nm. The UV visible spectra of Berberine chloride was shown in fig. 1. The calibration curve was constructed in the range of 2-10 µg/ml, and it was found to be linear with $r^2 = 0.996$ (fig. 2). The LOD and LOQ were calculated to be 0.215 and 0.652 respectively. The % RSD for Intraday and inter day precision were calculated for 0.449 and 0.604 respectively. The result of accuracy was 99.64-101.08 %. The summary of regression analysis and validation parameters was shown in (table 1). The content of total alkaloids in plant sample, formulation 1 and 2 were found in 0.271 %, 0.241 and 0.130 % respectively.

Table 1: Comparison of proposed method with existing methods

Plants	Method	Description	% yield	Ref
(<i>Berberis aristata</i> , <i>Solanum nigrum</i> , and <i>Piper longum</i>) <i>Hyptis spicigera</i>	Spectrophotometry	Dragendorff's reagent, yellow bismuth complex in nitric acid medium with thiourea, 435 nm.	0.195-0.567 %	[15]
	Gravimetric analysis	10% acetic acid in ethanol, conc. ammonium hydroxide solution	7.55 %	[16]
<i>Datura stramonium</i>	Acid base back Titration	Titrant: 0.1 N Sodium hydroxide Indicator: Methyl orange	0.23-0.54 %	[17]
<i>Nitraria schoberi</i>	Gravimetric	BCG, Yellow colour complex at pH 4.7, 470 nm	0.084-1.25%	[18]
<i>Nitraria schoberi</i>	Spectrophotometry	10% acetic acid in ethanol, conc. ammonium hydroxide solution	0.074-1.21 %	[19]
<i>Tinospora cordifolia</i>	Proposed method (spectrophotometry)	BCG, Yellow colour complex at pH 4.7, 415 nm	0.130-0.271 %	—

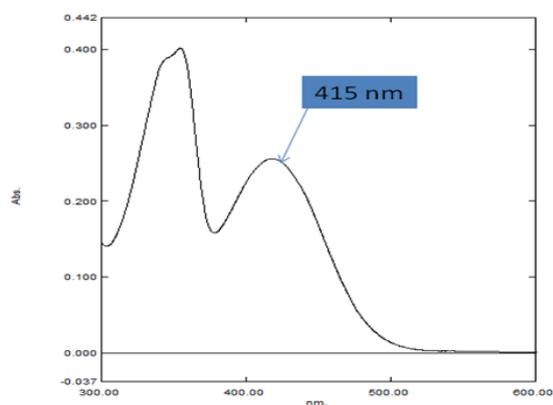


Fig. 1: UV-visible spectra of BER-H (Berberine chloride) standard solution

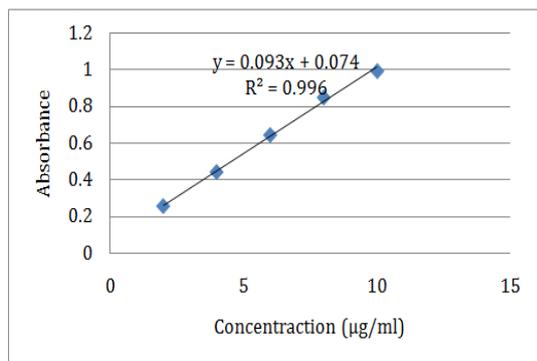


Fig. 2: Calibration curve of standard solutions (2-10 µg/ml)

Table 2: Summary of validation parameters

Parameters	Total alkaloids (Berberine chloride equivalent)
Linearity range (µg/ml)	2-10
Correlation coefficient (r^2)	0.996
Intraday Precision (%RSD)	0.449
Interday Precision (%RSD)	0.604
LOD (µg/ml)	0.215
LOQ (µg/ml)	0.652
%Recovery	99.64-101.08

The proposed method is simple, precise, sensitive and accurate and can be used as a part of routine quality control and standardization of plant materials of *T. Cordifolia* and its formulations containing alkaloids.

CONFLICTS OF INTERESTS

All authors have none to declare

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