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New extractive spectrophotometric method for estimation of total alkaloids in roots of *Cissampelos pareira*

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ABSTRACT

A simple and sensitive extractive spectrophotometric method was developed for estimation of total Alkaloids in root of *Cissampelos pareira* by using tropaeolin-'OOO'. The method is based on reaction between alkaloids and tropaeolin-'OOO' to form a charge transfer complex, which could be extracted in chloroform .A golden-red colour chromogen was formed which was measured at 485.5nm. It obeys beers laws in the range of 31-93μg/ml.Statistical analysis provide that the method was reproducible and selective and can be adopted as routine tool for standardization and quality control.

KEYWORDS

Total alkaloids;
Solvent extraction;
Tropaeolin 'OOO';
Beeberin;
Spectrophotometry.

INTRODUCTION

Roots of *Cissampelos pareira* Linn. ver. hirsuta, (Menispermaceae) Known as Laghupatha is an important drug in Ayurveda. Root of this plant is considered as stomachic. It also used in dyspepsia, diarrhea and cough. In Madagascar root is considered diuretic and antipyretic[4]. Root has been evaluated for various pharmacological activities such as, anti-inflammatory, anti-ulcer, antifertility and antioxidant[1]. The root is found to contain mainly Bisbenzyl isoquinoline type of alkaloids[5]. The Therapeutic efficacy of the drug can be attributed to its high alkaloidal content amongst which Bebeerine is the major one. In the present study, the total alkaloids from roots of *Cissampelos pareira* were estimated by extractive spectrophotometry using Tropaeolin 'OOO'. The method has its applicability in routine quality control for estimation of total alkaloids in root of *Cissampelos pareira*.

EXPERIMENTAL

Apparatus

All the spectral and absorbance measurements were made on Schimadzu 1700 model digital spectrophotometer with 10 mm matched quartz cells with stopper. A Toa-Dempa (Model HM-5B) pH meter with glass electrode was used to measure the pH of buffer.

Procedure for extraction of total alkaloids

The authenticated sample of *Cissampelos pareira* has been powdered and subjected for extraction with alcohol. The extracts has been concentrated under vacuum at low temperature and dissolved in 2.5% aqoues-acid solution, and partitioned with chloroform after making pH 8.5-9. Chloroform layer has been withdrawn and wash with water and subjects for concentration[6].

Full Paper

MATERIAL AND METHODS

Buffer preparation

Acetate buffer has been prepared by adding 5.4 gm of sodium acetate and 3.0ml of glacial acetic acid in 100ml of water and pH has maintained at 4.4.

Tropeolin-'OOO' preparation

Solution of Tropeolin -'OOO' (100µg/ml) has been prepared by adding 10mg of reagent in 100ml double distilled water.

Standard preparation (500µg/ml)

Beeberin (98%) is used as chemical marker and was purchased from Merck. 25mg of standard Beeberin has been dissolved in 3ml of conc HCl and volume was made up to 50 ml with double distilled water and refluxed for 1hr.

Sample preparation

Equivalent weight of Alkaloidal extract has been dissolved in 3ml of conc. HCl and made upto 50 ml with double distilled water and refluxed for 1hr and filter with watman no. 41.

Standard assay procedure

Into a series of 125 ml of separating funnels, different aliquots of standard solution (500µg/ml) from 0.25-2.0 ml, 5.0ml of buffer was added. Then 0.7 ml of Tropaeolin-OOO was added respectively[2]. The total volume of aqueous phase was adjusted up to 8.0 ml with distilled water. Then 4.0 ml of chloroform was added to all the separating fennels and contents were shaken for five minutes. The two layers were allowed to separate .The absorbance of the separated organic layer was measured at 485.5nm. The color was stable for one hour. The calibration curve was prepared by plotting the absorbance vs. concentration of the drug.

Determination of total alkaloids in root extracts *Cissampelos pareira*

Suitable aliquots of sample solution were taken for the estimation of total alkaloidal content. Color had been developed as the method described above and the absorbance was recorded at 485.5nm. The concentration of the total alkaloids was read from the calibration curve or computed from the regression equation and expressed in term of beeberin[3].

TABLE 1: Method validation parameters

Parameters	Results
λ_{max} (nm)	485.5
Linearity range (µg/ml)	31-93
Correlation coefficient(R^2)	0.9953
Slope	0.3924
Relative standard deviation (%RSD)*	±0.66
LOD(µg/ml)	0.021
LOQ(µg/ml)	0.065

*Precision (n=8)

TABLE 2: Recovery studies*

Amount of alkaloid present (µg/ml)**	Amount of std. Beeberin added (µg/ml)	Amount of total alkaloids found (µg/ml)	% Recovery
50	40.00	88.9 ±1.79	98.70%
50	50.00	98.2 ± 3.46	98.20%
50	60.00	107.7 ± 2.89	97.90%

**Total beeberine present is in alkaloid extract 27.9%, *Mean± Standard deviation

RESULTS AND DISCUSSION

The method has been validated for precision by repeating the experiment eight times with the same quantity of standard Beeberin. The accuracy of the method was determined by performing the recovery studies, by adding known amount of standard Beeberin to the alkalodal extract of *Cissampelos pareira*[5].

The calibration curve for Beeberin was found to be linear in range of 31-93µg/ml with a correlation coefficient of 0.9953. Precision of the method, expressed as relative standard deviation, was found to be $\pm$ 0.66 (TABLE 1) and the average percentage recovery was 93 to 96% (TABLE 2).

In conclusion, the proposed method is simple, sensitive, precise and accurate and can be adopted for routine quality control and standardization of roots of *Cissampelos pareira*.

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Full Paper

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