Vol. 2/2 (2002) 168 - 172

JOURNAL OF NATURAL REMEDIES

A new spectrophotometric method for the estimation of total alkaloids in the stem bark and seed of *Holarrhena antidysenterica* (Linn.) Wall. and in the Ayurvedic formulation, Kutajarishta

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Received 27 February 2002 ; Revised & accepted 6 April 2002

Abstract

Objective: To develop a spectrophotometric method for the estimation of total alkaloids in the stem bark, seed and formulations of *Holarrhena antidysenterica*. Materials and methods: A spectrophotometric method based on the formation of coloured complex between tropaeolin 'OO' reagent and alkaloids. Results: The developed method shows good accuracy and reproducibility. Conclusion: The method developed is simple, precise and accurate and can be adopted for the routine quality control purposes.

Key words: *Holarrhena antidysenterica*, conessine, spectrophotometric method

1. Introduction

Stem bark and root bark of *Holarrhena antidysenterica* (Linn.) Wall. (Family: Apocynaceae), known as kutaja and seeds known as indrajav in Ayurveda, have long been used in India for the treatment of dysentery and diarrhoea. They form part of many classical antidysenteric and antidiarrhoeal formulations, amongst which, the most widely used one is Kutajarishta (Ayurvedic Formulary of India, No.-1:11) (composition given in Table 1) [1]. The therapeutic efficacy of *H. antidysenterica* is due to the presence of alkaloids, of which, conessine is the major one. The total alkaloid content in *H. antidysenterica* stem bark varies from 0.22 to 4.2 % w/w [2,3]. The average content of total alkaloids in seeds is 1.825% w/w [2]. To the best of our knowledge, only titrimetric methods have been reported for the estimation of total alkaloids from *H. antidysenterica* [4-6]. In the present investigation, a simple spectrophotometric method was developed for the estimation of total alkaloids in stem bark, seeds as well as marketed formulations of *H. antidysenterica*.

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2. Materials and methods

2.1 Plant material and formulations

Stem bark and Seeds samples of *H. antidysenterica* were collected from the plants from different parts of the country, the details of which are as follows:

SB1- stem bark sample from Bangalore (Karnataka)

SB2- stem bark sample from Nagercoil (Tamil Nadu)

SB3- stem bark sample from Dediapada (Gujarat)

S1- seed sample from Gandhinagar (Gujarat)

S2- seed sample from Bhadam (Gujarat).

The samples were authenticated in our Pharmacognosy and Phytochemistry Department and voucher specimens were preserved. Samples of Kutajarishta formulated by 3 different manufacturers (K1, K2 and K3) were purchased from the local market.

2.2 Reference standard

Conessine was isolated from the stem bark of *H. antidysenterica* and its identity and purity was confirmed by melting point, IR spectroscopy, mass spectroscopy [7] and co-chromatography with standard conessine (a gift from Dr. K. K. Bhutani, NIPER, Chandigarh, India).

2.3 Chemicals

All the chemicals used were of analytical grade. Reagents used include acetate buffer of pH 4.6 (5.4 g sodium acetate and 2.66 ml glacial acetic acid in 100 ml of double distilled water), tropaeolin 'OO' solution (saturated solution of tropaeolin 'OO' in double distilled water) and acid reagent (1% v/v sulphuric acid in methanol).

2.4 Procedure for extraction of alkaloids

Alkaloids were extracted according to the method given by Bhutani *et al.* [3]. Briefly, 0.5 g powder of stem bark or seed was extracted with 50 ml of 5% hydrochloric acid by overnight maceration. The extract was filtered and the marc was further extracted with 5% hydrochloric acid (2 x 20 ml) and filtered. The filtrates were pooled, basified with liquor ammonia (pH~9.0) and extracted with chloroform (5 x 25 ml). The combined chloroform extract was concentrated, dried over anhydrous sodium sulphate and the volume was made up to 100 ml with chloroform.

For the extraction of alkaloids from Kutajarishta, 5 ml of Kutajarishta was mixed with 15 ml of 5% ammonia solution and extracted with chloroform (4 x 25 ml). The combined chloroform extract was dried over anhydrous sodium sulphate and volume was made upto 100 ml with chloroform.

Table 1
Formula of Kutajarishta

Ingredients	Part used	Quantity
Holarrhena antidysenterica (Linn.)	Root bark	4.800 kg
Vitis vinifera Linn.	Dry fruits	2.400 kg
Madhuca indica J. F. Gmel.	Flowers	0.48 kg
Gmelina arborea Linn.	Stem bark	0.48 kg
Water for decoction	—	49.152 L
Molasses	—	4.800 kg
Woodfordia fruticosa Kurz.	Flowers	0.960 kg

Table 2
Method validation parameters

Parameters	Results
Linearity range	0.5 - 4.0 μg/ml
Correlation coefficient	0.998
Slope	0.003874
Intercept	- 0.0582
Precision (n=5; % RSD)	± 1.02
Accuracy	98.1 %

2.5 Calibration curve

For the preparation of calibration curve, standard solution of conessine (100 μg/ml) was prepared in methanol, aliquots of 0.25 - 2 ml were taken and colourimetric analysis was carried out following the method of Haussler [8]. In brief, the method is as follows: Solvent from the aliquots was evaporated at room temperature and the residue was dissolved in 1 ml of methanol. 5 ml of acetate buffer and 3 ml of tropaeolin 'OO' solution were added to it and mixed well. The complex thus formed was extracted in chloroform (3 x 15 ml). The chloroform extract was dried over anhydrous sodium sulphate, transferred to a 50 ml volumetric flask containing 3 ml of acid reagent and the volume was made up with chloroform.

The colour developed was measured at 545 nm against blank, using double beam UV-VIS spectrophotometer (Jasco-7850). Calibration curve was prepared by plotting concentration of conessine versus absorbance.

2.6 Estimation of total alkaloids in the stem bark, seed and formulation of H. antidysenterica

For the estimation of total alkaloids in stem bark and seed samples and in the formulation, suitable aliquots of sample solutions were taken and colour was developed as per the method described above (refer 2.5). Absorbance of the coloured solution was recorded at 545 nm. The amount of total alkaloids in the stem bark and seed samples and Kutajarishta samples was calculated using calibration curve. The content of the total alkaloids in the different samples was expressed in terms of conessine.

2.7 Method validation

The method of analysis was validated for precision and accuracy. The method was checked for precision by repeating the experiment 5 times with the same quantity of conessine. The accuracy of the method was determined by performing the recovery study at two levels, by adding known amounts of conessine to the stem bark sample.

3. Results and discussion

The proposed method is based on the reaction between alkaloids and tropaeolin 'OO' to form a charge transfer complex, which can be extracted in chloroform or dichloromethane,

Table 3
Percent recovery studies

Amount of total alkaloids present (μg)	Amount of conessine added (μg)	Amount of total alkaloids found (μg)*	% Recovery
99	50	146.4 ± 3.47	98.27 ± 2.32
99	100	194.9 ± 3.63	97.96 ± 1.82

* Mean ± standard deviation; n = 3

Table 4
Content of total alkaloids in the stem bark and seed samples of *H. antidysenterica* and in Kutajarishta samples

Samples	Content of total alkaloids*
Stem bark	(% w/w)
SB1	2.13 ± 0.168
SB2	1.72 ± 0.110
SB3	3.02 ± 0.087
Seed	(% w/w)
S1	2.46 ± 0.075
S2	1.43 ± 0.028
Kutajarishta	(mg/ml)
K1	0.38 ± 0.023
K2	0.20 ± 0.010
K3	0.66 ± 0.012

* Mean ± standard deviation; n = 3

followed by its reaction with acid reagent to give a purple coloured chromogen with λ max of 545 nm [8-14].

Various parameters affecting the colour development such as pH and amount of acetate buffer, amount of tropaeolin 'OO' solution, amount of acid reagent and time span stipulated for various stages of the reaction were optimized. The chromogen formed was found to be stable up to 3 h. The calibration curve for conessine was found to be linear over the range of 0.5 to 4.0 μg/ml with a correlation coefficient of 0.998. Precision of the method, expressed as relative standard deviation, was found to be 1.02% (Table 2) and the average percentage recovery was 98.11% (Table 3).

The method was applied for the estimation of total alkaloids in the stem bark and seed samples of *H. antidysenterica* and in three market samples of Kutajarishta. The results of the analysis are shown in Table 4.

In conclusion, the proposed method is simple, sensitive, precise and accurate and can be used as a part of routine quality control of seed and stem bark of *H. antidysenterica* as well as formulations. Since other alkaloids also form coloured complex with tropaeolin 'OO' [8-14], this method is not specific for the alkaloids of *H. antidysenterica* and hence, is applicable only to those formulations which have only *H. antidysenterica* and not any other alkaloid containing drug.

4. Acknowledgement

We thank Industries Commissionerate, Government of Gujarat, for the financial aid towards instrumentation facility.

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