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Spectrophotometric Determination of Total Alkaloids in *Peganum harmala* L. Using Bromocresol Green

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Abstract: A simple spectrophotometric method is described for determination of total alkaloids based on the reaction with Bromocresol Green (BCG). A yellow complex forms and is easily extractable by chloroform at pH 4.7. The absorbance of the complex obeys Beer's law over the concentration range of 4-13 µg atropine per mL of chloroform. This procedure can be carried out in the presence of other compounds without interference.

Key words: BCG, *Peganum harmala* L., total alkaloids, spectrophotometry

INTRODUCTION

The *Peganum harmala* L. (Syrian rue) is a wild-growing flowering plant belonging to the Zygophyllaceae family and is found abundantly in Middle East and North Africa (Zargari, 1999). From ancient times, it has been claimed to be an important medicinal plant. Its seeds are known to possess hypothermic and hallucinogenic properties (Lamchouri *et al.*, 1999; Kuhn and Winston, 2000). It has been used traditionally as an emmenagogue and an abortifacient agent in the Middle East and North Africa (Abdel-Fattah *et al.*, 1997). There are several reports in the literature indicating a great variety of pharmacological activities for *Peganum harmala* L. such as anti-bacterial, antifungal and MAO-inhibition (Saleem *et al.*, 2001). It has also been known to interact with β_2 -adrenoceptor subtypes (Goghataei *et al.*, 2003) and have hallucination potency and be effective in the treatment of dermatosis (Abdel-Fattah *et al.*, 1995) and cancer (Shi *et al.*, 2001). The pharmacologically active compounds of *P. harmala* are several alkaloids, which are found especially in the seeds and the roots. These include β -carbolines such as: harmine, harmaline (identical with harmidine), harmalol and harman and quinazoline derivatives: vasicine and vasicinone. The alkaloidal content of the unripe seeds is less than the ripe ones (Du *et al.*, 1997).

The objective of this study was to describe a direct, simple and sensitive spectrophotometric method for the determination of total alkaloids in the seeds of *Peganum harmala* L., based on the reaction with bromocresol green.

MATERIALS AND METHODS

Bromocresol Green Solution (1×10^{-4})

Warm 69.8 mg bromocresol green with 3 mL of 2N NaOH and 5 mL distilled water until completely dissolved and dilute to 1000 mL with distilled water.

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Phosphate Buffer (pH 4.7)

Adjust pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Atropine Standard Solution

Dissolve 1 mg pure atropine (Sigma Chemical Co.) in 10 mL distilled water.

Plant Material

The seeds of Syrian rue, collected from local market of Tehran province, in May 2003, were used in this investigation. The seeds were identified in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

Preparation of Standard Curve

Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 mL) of atropine standard solution and transfer each to different separatory funnels. Add 5 mL pH 4.7 phosphate buffer and 5 mL BCG solution. Shake mixture with 1, 2, 3 and 4 mL of chloroform. The extracts were collected in a 10 mL volumetric flask and then diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Extraction Preparation

The dry seeds of Syrian rue (100 g) were grinded and then extracted with methanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness. A part of this residue was dissolved in 2 N HCl and then filtered. One milliliter of this solution was transferred to a separatory funnel and washed with 10 mL chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 mL BCG solution and 5 mL phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3 and 4 mL chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 417 nm vs. similarly prepared blank.

RESULTS

A yellow-colored complex with a maximum absorption at 470 nm was developed (Fig. 1). It was completely extractable by chloroform at pH 4.7. A calibration curve was plotted for various

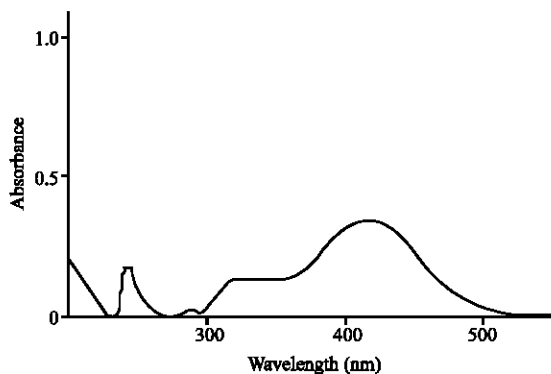


Fig. 1: Absorption spectra of the atropine-BCG complex at pH 4.7

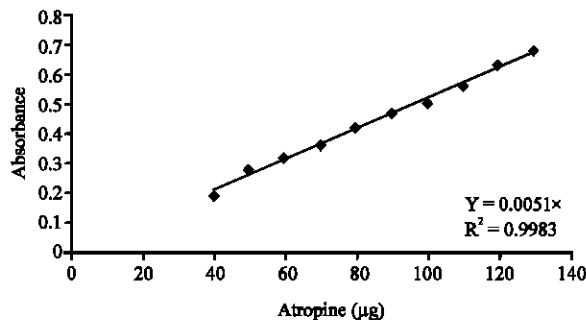


Fig. 2: Variation of the absorbance vs. the atropine concentration at 470 nm

concentrations of atropine (Fig. 2). Beer's law was followed over the concentration range of 4-13 µg atropine per mL of chloroform. The effects of temperature and pH were studied. A pH of 4.7 gave optimum results and different temperatures had no effect on complex formation and extraction. The complex was very stable in chloroform and began to fade slowly only after 10 days. Before the extraction, the mixture was put in a boiling water bath for 3 min the absorbance did not change after extraction with chloroform.

DISCUSSION

A few methods with different sensitivities have been developed for the determination of alkaloids in plant materials for example gravimetric and titrimetric methods. But these methods lack the adequate sensitivity and have some problems. As with most gravimetric methods, the residue obtained was found to be impure since more than one spot was revealed by TLC. The titrimetric assay suffers from the disadvantage that the end-point is masked by the color of the extract. On the other hand, there is no constant method applicable for all alkaloids. Methods with high sensitivity such as HPLC, are not routine methods for the determination of the total alkaloids and these methods are very costly and need special equipment. Spectrophotometric determination of total alkaloids with bromocresol green is a simple and sensitive method and do not need very special equipment. The proposed procedure has the advantage of being less time consuming, with the assay requiring an average of 1 h.

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