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Analysis of Anthraquinone in *Aloe barbadensis* by using RP-HPLC

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Abstract

Aloe barbadensis is a herb possessing medicinal value commonly found in the Indian subcontinent. Quantitative determination of anthraquinone, was undertaken to provide an easy and simple analytical method, which can be used as a routine quality control method. RP-HPLC was performed using methanol and water as mobile phase. The detection and quantification was performed at a wavelength of 240 nm. Linearity of detector response for anthraquinone was between the concentrations 0.05% to 0.2%.

Keywords: HPLC, anthraquinone, *Aloe barbadensis*

Abbreviations: RP-HPLC- Reversed phase- high perferformance liquid chromatography, H_2O-Water HPLC grade, AQ- Anthraquinone

Introduction

Aloe barbadensis is a valuable herb in Ayurvedic medicine, and as such, was used and cultivated for centuries in India. In Ayuervedic and the traditional medicine of India, *Aloe barbadensis* is used internally as laxatives, antihelminthic, hemorrhoid remedy and uterine stimulant as menstrual regulator[1] for wound healing, conjunctivitis and as a disinfectant and laxatives[2,3].

Aloe barbadensis gel is an active ingredient in hundreds of skin lotions, sun blocks and cosmetics[4]. The gel used in cosmetics has been claimed that it has similar antiaging effects to Vitamin A derivatives[5].

The biological activities of quinones, especially of the dominant Anthraquinone have been studied extensively. Notable activities reported for this compound, include antiinflammatory, anticonvulsive, antitumor and antioxidant properties[6]. Claiming adaptogenic effects, Aloevera products are available throughout the world.

These compounds are specific for the Liliaceae family, and in particular, for the genus *Aloe barbadensis*, and thus they are used as marker compounds. The biological activities of phenols, especially of the dominant Anthraquinone has been studied extensively and reported from the leaves of the species of *Aloe* barbadensis[7]. The number of analytical reports for the determination of anthraquinone is comparatively small. Besides a TLC method for the quantification of anthraquinone, a few HPLC methods are described in literature[8]. Most of them showed disadvantages, as either the acetylation of Anthraquinone is required prior to analysis[9], the separation time is long[10] or the compounds are not baseline separated and elute, more or less with the injection peak[11]. A part of our efforts was to develop a HPLC method suitable for direct determination of Anthraquinone in *Aloe barbadensis*, and to investigate the distribution of this compound in different plant parts.

Material and Methods

Authenticated *Aloe barbadensis* plant materials were collected from St. Thomas College, Bhilai and botanically identified. The leaves of *Aloe barbadensis* were used for HPLC analysis. Standard Anthraquinone was procured from the Hi-media Mumbai.

The 5% methanol extracts of leaves of *Aloe barbadensis* plant, were prepared by soaking the respective plant material separately for 18 h, in methanol and with soxlet extractor. The extracts were filtered through Whatmann filter paper No.1, using high-pressure vacuum pump.

One milligram of standard Anthraquinone, was dissolved in 1 ml of acetonirile. Three additional calibration levels were prepared by diluting this solution with methanol, to obtain 0.05%, 0.1%, 0.15% and 0.2% concentrations, for studying the linearity. The precision of the method was also studied, by injecting a sample solution of concentration 0.2% five times, and finding out the standard deviation and coefficient of variation.

A gradient HPLC (Perkin Elmer) with Merck C-18 select Bondapak (4.0 mm x125 mm) column was used. The HPLC system was equipped with software. The mobile phase components acetonitrile: water were filtered through 0.2 µ membrane filter before use, and were pumped from the solvent reservoir at a flow rate of 1ml/min, which yielded column backup, pressure of 215-250 kgf/cm^2. Then 20 µl sample was injected into a column which was eluted isocratically with H_2O / methanol (63:33 V/V). The column temperature was maintained at 27°. Rheodyne syringe (Model 7202, Hamilton) was used for injecting 20 µl of respective samples.

Results and Discussion

Standard Anthraquinone solutions of 0.05%, 0.1%, 0.15% and 0.2% concentrations were analyzed for studying the linearity, and the area count obtained for these solutions are presented in Table 1. Anthraquinone showed good linearity in the concentration range of 0.05% - 0.2%, with a correlation coefficient of 0.993. The

precision of the method was also studied by injecting a single sample solution five times (Table 2.), and finding out the standard deviation and coefficient of variation. The standard deviation and coefficient of variation were found to be 0.526 and 0.858. Low value of standard deviation and coefficient of variation are indicative of high precision of the method.

The HPLC chromatogram of standard Anthraquinone at an optimum wavelength of 240 nm, showed a mean area (Table 2.) of 7729045, at a mean retention time of 2.073 min (Fig. 1). The recovery value of standard Anthraquinone was 94.4%. The HPLC chromatogram of leaf of *Aloe barbadensis* corresponding to standard Anthraquinone was showed at a retention time of 2.356 min, with an area of 6438274 at a wavelength of 240 nm (Fig. 2). The variation in retention time of peak of Anthraquinone in chromatograms of *Aloe barbadensis,* may be due to the presence of other chemical constituents. The quantitative evaluation of Anthraquinone in leaf of *Aloe barbadensis,* was 4.270%. High content of Anthraquinone in leaf was reported from Lunis *et al*., 1990[12].

The proposed method can be used to standardize *Aloe species* on the basis of Anthraquinone as a marker compound, and is helpful for scientific as well as commercial application.

Table 1: Area counts for standard anthraquinone.

Concentration of Anthraquinone (%)	Area counts
0.05	2245568
0.1	4583699
0.15	6587109
0.20	7805457

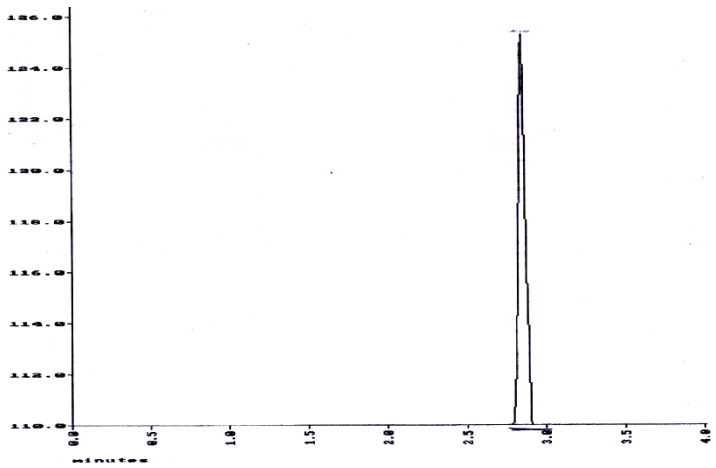

Figure 1: HPLC chromatogram of standard anthraquinone.

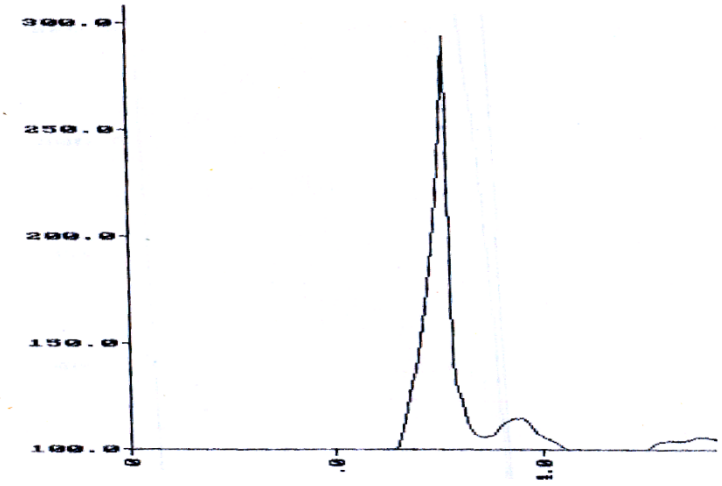

Figure 2: HPLC chromatogram of *Aloe barbadensis* leaf.

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