

Monitoring the modifications of *Aloe vera* by high performance liquid chromatography

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Abstract. *Aloe vera* is a widely known and studied plant due to its therapeutic properties. The therapeutic properties exhibited by the *Aloe vera* exudates have been associated to the presence of certain compounds, such as, chromones, anthrones and anthraquinones. In this study, we have identified, and monitored by reversed phase high performance liquid chromatography (HPLC), six major compounds present in the *Aloe vera* exudates. The chromatographic profiles were followed in the course of time and at different wavelengths. This monitoring allowed us to verify the constitution and modifications of the samples, in order to identify the main changes responsible for the chemical degradation and loss of properties exhibited by the exudates over time.

Keywords: *Aloe vera* exudate, reversed phase HPLC.

INTRODUCTION

Aloe is a plant that belongs to the *Asphodelaceae* family and to the *Aloioideae* sub-family, being present in nature in more than 600 sub-species [1, 2]. This plant is mainly found in the South and West of Africa, Central America and Asia. It has been used for medical purposes for more than 3000 years [3] due to its laxative, anti-inflammatory, anti-septic, stabilizer, and wound healing properties, among others [4, 5]. The most common species of the *Aloe* plant are *Aloe aborescens*, *Aloe chinensis* and *Aloe vera*, being the later, the most biologically active [4], and the subject of the present study.

Aloe vera (sin. *Aloe barbadensis* Miller) is a fleshy plant native from North Africa, specially adapted to hot and dry environments. The *Aloe vera* leaf can be divided into two distinct parts: (1) the mesophyll, which corresponds to the outer green section (including the vascularisation channels) and (2) the parenchyma (the inner section), which gum is more translucent and where the *Aloe vera* gel is extracted from [4, 6]. The gel is commonly known by the presence of phenolic substances, such as, chromones, anthraquinones, anthrones, and others. Moreover, it is also the high content in water presented by this plant that provides an exceptional environment for the maintenance of the characteristic properties of *Aloe* [6].

Chromones are heterocyclic compounds with wide biological functions. The common forms of the chromones found in nature, as well as, their derivatives are notorious for biocide, pharmacological and anti-oxidant reasons [7]. The most common chromones in the *Aloe vera* gel are usually derivatives from 8-*G*-glucosyl-7-hydroxi-5-methyl-2-propyl-4-chromone with other variations generally descendent from the oxidation of the propyl group, methylation of the C7-hydroxi group and sterification of the glucose moieties. Some examples of *Aloe vera* exudate chromones are Aloesin, Isoaloeresin D, Aloesone and Aloesol [6]. Anthraquinones are aromatic compounds derived from anthracene and are characterized by their anti-inflammatory, wound healing, analgesic, anti-pyretic, anti-microbial and anti-tumour properties [8]. The main anthraquinones in the *Aloe vera* comprise Aloe-emodin and

chrysophanol. Anthrones are tricyclic aromatic compounds with anti-inflammatory and anti-psoriatic properties [9]. Within this class, we can find Aloin A, Aloin B, Aloinoside A and Aloinoside B [10]. Alves *et al*, [8] reported that the most frequently found C-glycoside anthrone in *Aloe vera* is Aloin A (barbaloin) and that the glycosides moieties cleavage in the intestinal flora results in Aloe-emodin (anthraquinone) with laxative properties. Thus, it is the specific combination of these particular compounds that turns *Aloe vera* in such a pharmacologically and cosmetically interesting plant.

In this study, the *Aloe vera* gel extracted from the leaf was evaluated and monitored by HPLC over 15 days. The *Aloe vera* compounds were firstly identified as reported by Okamura *et al*, [2], Saccù *et al*, [3], Zonta *et al*, [5] and Park *et al*, [11]. Saccù *et al*, [3] have characterized several *Aloe barbadensis* and *Kenya aloe* exudates by HPLC. Park *et al*, [11] used this method to identify and quantify over thirteen *Aloe vera* and *Aloe arborescens* phenolic compounds. Accordingly, they have used a water/methanol gradient as the mobil phase, at a flow rate of 0.7 ml/min, with readings at a wavelength of 293 nm. For comparison, we have carried out a complementary HPLC analysis followed at 220 nm, and using a water/acetonitrile gradient at a flow rate of 1ml/min.

MATERIALS AND METHODS

Commercial Products

The *Aloe vera* plant used in this experimental study was acquired in Viveiros de São Jorge (São Jorge, Portugal). The acetonitrile used was achieved from Lab-Scan (Dublin, Ireland).

HPLC equipment

The HPLC equipment used in this experiment is based on a Agilent 1100 Series manual injection system (G1328 A), a quaternary pump (G1311 A), a degasser (G1322 A) and a UV-Visible detector 1100 Series variable wavelength (G1314 A), all from Agilent Technologies. The samples were injected in a valve with a 20 µL loop and the chromatograms were obtained through the Instruments software (Hewlett Packard®). The compounds were separated by using a reversed phase column Hypersil BDS-C18 (5 µm) with dimensions of 4.0 x 250 mm from Agilent Technologies.

Sample preparation - HPLC

Aloe vera leafs were extensively washed with sterilized water. The *Aloe vera* sample was obtained through direct extraction from the leaf parenchyma. Subsequently, the parenchyma extraction was held under controlled atmosphere conditions, and the following vacuum filtrations were performed by gradually and selectively filtering the samples through filters with different porosities of 35-40 µm, 2 µm and 0.22 µm. Filtered samples were kept under vacuum and at 5°C. To avoid any kind of sample contamination, all glass materials were properly sterilized by autoclavation.

Reversed Phase HPLC analysis

The HPLC analysis was executed according to the method described by Saccù *et al*, [3] and using the following conditions: mobil phase: water [A] and acetonitrile [B] (v/v): 74 – 16 % (A – B) (12 min, isocratic); 66 – 33 % (25 min, linear); 40 – 60 (13 min, linear); 0 – 100 % (10 min, linear), followed by 10 min of isocratic gradient for column cleaning. The chromatography was performed at room temperature (25°C), at a flow rate of 1 ml/min and with the UV-Visible detector set at 293 nm. The other wavelengths tested were: 220 nm [3, 5] and 293 nm [11], and a randomly chosen midpoint of 250 nm.

RESULTS AND DISCUSSION

Optimization of the HPLC analysis

The preliminary HPLC experiments were performed with detection at several wavelengths. The results obtained were equivalent, solely varying in the absorbance intensities. Thus, taking this into account, the wavelength of 293 nm was chosen for quantification, due to its enhanced peak resolution.

Identification of compounds

The elution gradient of the reversed phase HPLC allowed a hydrophobicity-based separation of the several compounds present in the freshly obtained *Aloe vera* exudates. The separation was successfully executed in 32 minutes. The *Aloe vera* chromatographic profile collected on the first day of monitoring is shown in Figure 1. It is possible to identify five major peaks from the chromatogram. According to the work reported previously [2, 3, 4], while compounds 1 and 2 are likely to be aloesin [2, 3] and 8-C-glucosil-7-O-metil-(S)-aloesol [2, 4], as judged by their less hydrophobic behaviour; compounds 3, 4 and 5 are expected to be aloin and aloinosides B and A, respectively, as judged by the hydrophobic character characteristic of these compounds [3].

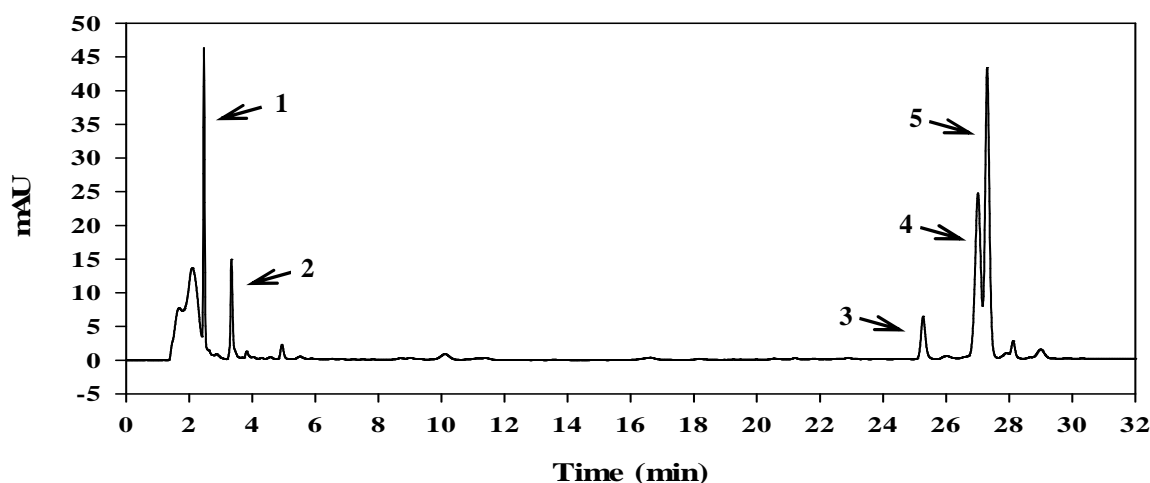


Figure 1: Reserved phase HPLC chromatogram of the *Aloe vera* sample detected at 293 nm, on the first day of analysis. The major peaks present in the sample are labeled from 1 to 5.

The monitoring proceeded during the next 15 days. The chromatograms obtained showed a decrease of some of the peaks, pointing towards the chemical modification of the initial sample. These data are in agreement with the results reported by Zonta *et al.*, [5] where the authors detected, after several days of monitoring, a considerable reduction in some of the peaks coupled to the degradation of aloin and of the aloinosides. Concomitantly, the intensity of other peaks, such as compound 1 (Fig. 1), has increased with time, probably due to the products of degradation of aloin and of the aloinosides. Figure 2 shows the profiles obtained on 1st, 5th, 10th and 15th day of analysis after extraction of the exudates.

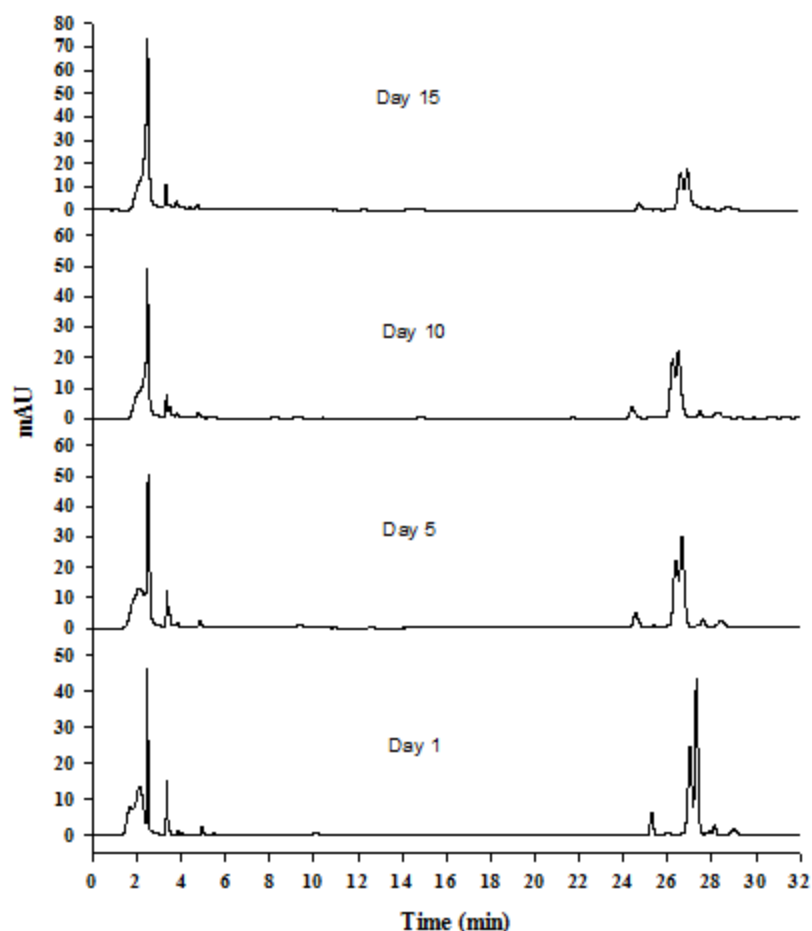


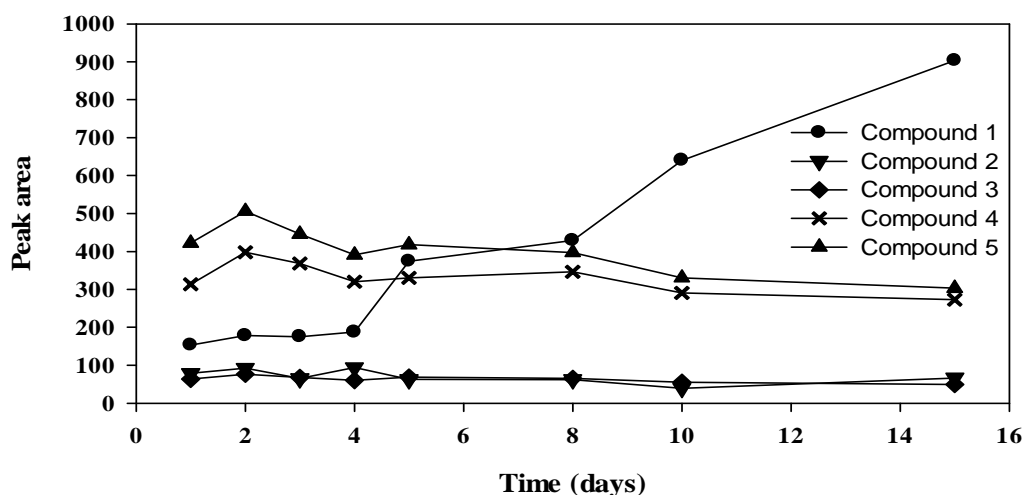
Figure 2: HPLC profiles of *Aloe vera* at different days after extraction of the fresh exudate.

Quantitative results

The area of the identified peaks was also followed through time. Table 1 and Figure 3 present the results obtained upon integration of the five major peaks. It is possible to verify that, while compound 1 is the most prominent, presenting the highest areas; compounds 2 and 3 present the lowest areas. Moreover, the area of compound 1 shows an increase with time. This increase may be associated to the formation of degradation products, due to the chemical modification of the sample. Compounds such as aloins, which are converted to aloe-emodin through oxidation processes have already been pointed out as some of the degradation products of the *Aloe vera* exudates [2, 4]. On the contrary, while compound 2 remains oscillatory; compounds 3, 4 and 5 present a general decrease in peak area with time. The decrease in the area of the most hydrophobic compounds, coupled to the concomitant increase in the peak area of compound 1, corroborates the suggestion that the *Aloe vera* sample is suffering modifications with time, probably due to the oxidation of the aloins and of the aloinosides.

Table 1: Peak area determined for each compound along time.

Day	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
1	153.08	78.36	63.48	313.32	422.27
2	177.83	91.94	76.57	397.66	506.19
3	175.54	64.93	67.35	367.93	445.74
4	187.27	93.87	59.73	319.75	391.12
5	373.89	62.64	68.27	330.52	417.89
8	428.68	62.09	65.43	346.05	379.15
10	639.89	39.75	55.13	290.55	330.65
15	902.99	66.16	49.24	272.91	303.26

**Figure 3:** Changes of peak area with time. The plot shows the peak areas determined for each compound at each day of monitoring.

CONCLUSIONS

In conclusion, the HPLC method is useful for the qualitative and quantitative monitoring of the *Aloe vera* samples. The *Aloe vera* exudates do suffer degradation over a period of time of 15 days. After this period, most of the peaks decrease in absorbance, being the reduction more pronounced for the peaks associated to the more hydrophobic compounds, presenting higher retention times. Future work includes the effective identification and characterization of the compounds present in the *Aloe vera* sample, as well as, the development of a biomaterial interface that would preserve the therapeutic properties of *Aloe vera* for longer times.

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