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Quantification of Aloin in *Aloe barbadensis* Miller Leaf Gel and Latex from Selected Regions of Kenya

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Abstract

Aloe barbadensis Miller (*A. barbadensis*) has been reported for use in the traditional management of malaria in Kenya. Aloin (an anthraquinone-C-glycoside) is one of the therapeutic molecules in *Aloe* species that is responsible for their antimalarial effect. However, there is no report on the aloin content of *A. barbadensis* leaves latex and gel from Kenya. This study, therefore, isolated and quantified aloin levels in Phosphate Buffered Saline (PBS) extracts of *A. barbadensis* leaf gels and latexes sampled from Kisumu, Elgeyo-Marakwet and Baringo Counties of Kenya. Aloin was isolated by preparative thin layer chromatography and then subjected to thin layer chromatography and quantified using High-Performance Liquid Chromatography (HPLC). Results showed that the highest aloin content of 237.971 ± 5.281 mg aloin/g DW was for dry latex from Elgeyo-Marakwet followed by those from Baringo (198.409 ± 2.000 mg aloin/g DW) and then Kisumu (40.760 ± 0.088 mg aloin/g DW). Latexes had comparatively low aloin contents, and followed the order Kisumu > Baringo > Elgeyo-Marakwet. The HPLC method validation was satisfactory and exhibited adequate linearity, repeatability and accuracy. The HPLC method developed for identification and quantification of aloin in *A. barbadensis* leaves had high sensitivity, is specific, and the mobile phase systems and sample preparation method are simple. This can be used for quality control of Kenyan *Aloe* extracts. The results indicated intraspecific variation in aloin content of *A. barbadensis* leaf gels and latexes from different regions of Kenya.

Subject Areas

Analytical Chemistry, Medicinal Chemistry

Keywords

Aloe vera, Aloin, High-Performance Liquid Chromatography, Kenya

1. Introduction

The use of medicinal plants as the source of primary health care has continued to gain momentum in developing countries [1]. This is in part due to limited access to health services, poverty and the mental picture that herbal medicines are readily available, effective, affordable, and widely accepted across cultures [2]. More than 80% of the global population subsists on traditional medicine for their primary health care, and this has been a veritable source from which various modern medicines have been discovered [3]. Hence, rigorous ethnobotanical surveys and scientific validation studies of medicinal plants can enhance the discovery of novel lead molecules that can be used to counter the current multidrug resistance by parasites, tumor cells and microorganisms [4] [5] [6].

Aloe barbadensis Miller (*A. barbadensis*) is a cactus-like perennial herb that is traced to have originated in Arabian Peninsula and North Africa and spread to other areas of the globe as an invasive alien species [7] [8] [9]. Its leaves (latex and mucilaginous gel) are the most used part in traditional medicine, food, cosmetic and pharmaceutical sectors [7] [10] [11]. In Kenya, *A. barbadensis* leaves have been used in Kenya for the phytotherapeutical management of malaria [12] [13].

According to a recent survey by Omara [14], there is a paucity of data on the antimalarial activity and phytochemical composition of *A. barbadensis* used in antimalarial phytotherapy in Kenya. Aloin, an anthraquinone-C-glycoside presented in this species has antimalarial activity, but it is extremely variable among different *Aloe* species, contingent on the growing conditions of the plants. In addition, the quantification of aloin in various solvent extracts has been unsuccessful owing to its chemical instability. Conventionally, a range of analytical solvents and techniques have been used to extract and quantify anthraquinones (including aloin) from plant matrices. The techniques used include capillary electrophoresis [15] and High-Performance Liquid Chromatography (HPLC) with ultraviolet-visible detection [16]-[21]. The solvents used include water, isopropyl alcohol [20], phosphate buffered saline [22], ethyl acetate [23], ethanol [16] and methanol [24], though the methanol is more frequently used. The utilization of an ultrasensitive ionic liquid microextraction procedure has also been exploited [25]. Based on the best extraction solvent established by Sánchez-Machado *et al.* [22] and a study in our laboratory [26], this investigation aimed at quantifying aloin levels in phosphate buffered saline extracts of latex and gel of *A. barbadensis* leaves from Kisumu, Elgeyo-Marakwet and Baringo Counties of Kenya using HPLC.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used in this study, unless otherwise stated, were of analytical and HPLC grades, sourced from Merck and Sigma Aldrich.

2.2. Collection, Authentication and Preparation of Samples

Aloe barbadensis leaves were collected between May 2021 and June 2021 from plants in their natural habitats in Kisumu, Elgeyo-Marakwet and Baringo Counties, representing the hot, moderately hot and cold regions of Kenya, respectively (**Table 1**). They were identified and authenticated by a taxonomist at the Department of Biological Sciences, Moi University, Kenya where voucher samples (CKN 010521, CKN 020521, CKN 030521) were deposited. Voucher samples were also deposited at the Herbarium of University of Eldoret, Eldoret, Kenya for future reference.

Extraction was done according to the method used by Sánchez-Machado *et al.* [22]. Fresh latex, fresh gel, dry latex and dry gel were extracted and stored separately. The leaf latex of *A. barbadensis* was obtained by cutting the leaves transversally near the base using a knife and arranging them concentrically. They were drained for 30 minutes into a clean beaker. Then the collected latex was left in open air for 3 days to allow complete evaporation of water [22] [27].

The resulting leaves were washed under running tap water and then distilled water. They were surface sterilized with 10% sodium hypochlorite to prevent microbial contamination. Fresh gel from the leaf skin was removed and the inner and middle layers were scraped [28] and homogenized in a blender. For dry samples, fresh gel was dried at 40˚C for 24 hours in an oven while the fresh latex was dried at room temperature (25˚C) for 24 hours. The dried samples from both gel and latex were separately extracted. The crude extracts thus obtained were kept at 4˚C in a refrigerator until commencement of analysis [22].

Measured 5 mg of dry latex were dissolved in PBS at pH 3 and topped up to the 5 mL mark. A total of 50 mg of dry gel was prepared by dissolution in PBS to make a 10 mL solution. All samples were sonicated on a digital Ultrasonic Cleaner (LMUC Series, Model-USBT-6-Liters) for 10 minutes and filtered through 0.45 µm pore size nylon membrane filters [22] [29]. The extraction process was replicated twice. All the extracts obtained were freeze dried using a FD5-serries freeze dryer (MRC Scientific Instruments).

Table 1. Sampling location of *A. barbadensis* plants where leaves were sampled in Kenya.

County	Sampling site	Geographical position system location
Kisumu	Katho (Upper Bwanda)	0˚11'44.5"S 34˚51'46.8"E
Elgeyo-Marakwet	Birweto	0˚32'34.32"N 35'26.17"E
Baringo	Timboroa	0˚18'59.3"N 35˚20'05.6"E

2.3. Isolation of Aloin from *A. barbadensis* Leaf Latex and Gel

Isolation of aloin using precipitation method gave very low yields. Thus, Preparative Thin Layer Chromatography (PTLC) method by Geremedhin *et al.* [27] was used with some modifications. Calcium sulphate (15%) mixed with silica (85%) were slowly dissolved in distilled water. The mixture was then poured onto a glass plate and spread with a metric ruler. The plate was put in a shaker to perfect the preparation then it was sun dried naturally and later activated in the oven at 120˚C for 2 hours.

Isolates were obtained from the latex extracts using PTLC prepared by coating the glass plate with silica gel type G (Size: 10 - 40, Sigma-Aldrich, USA) at 0.5 mm thickness in the laboratory of Pharmacy Department at Mbarara University of Science and Technology, Uganda. The dry latex was dissolved in methanol and applied directly as a band to the coated PTLC (20 cm × 20 cm) over one side of the plate. Ethyl acetate and methanol mixture (7:3) were used as a solvent system to develop the chromatogram. The isolates were checked for their purity using silica gel over TLC plate (0.25 mm thickness).

The chromatographic zones were visualized using ultraviolet light at 254 nm and 366 nm. After visualization, the chromatographic zones were coded in ascending order of retention factor (Rf) values. Then, each band was carefully scrapped off separately from the plate and dissolved in methanol and chloroform (1:1), filtered and concentrated [27].

2.4. Thin Layer Chromatography Finger Printing of the Extracts

A portion of the powder (0.1 g) was subjected to silica chromatography column using 75% water acetic acid: 25% ethyl acetate (v/v) to afford clean aloin.

2.5. Quantification of Aloin

Aloin in the extracts was quantified using HPLC. The analysis was performed on an UFLC Shimadzu HPLC system (Prominence Model, Tokyo, Japan) at the Analytical and Pharmaceutical Laboratory of Mbarara University of Science and Technology, Uganda. The HPLC system comprised of an LC-20AD pump, a Phenomenex Luna C18 column (250 × 4.6 mm × 5 µm), temperature-controlled sample trays, an online degasser (DGU-20A5R) and UV detector.

Aloin quantification was carried out using the external standard method as described by Meng *et al.* [30] with some modifications. A stock solution of 1 mg/mL of aloin standard was prepared by weighing 1 mg and dissolving in 1 mL of HPLC grade methanol. From this, working solutions of 0.5, 5, 10, 20, 50, 100 and 150 µg/mL of aloin were prepared. Triplicate injections of 10 µL of each concentration were run to establish the calibration curve and ensure the linearity.

A reversed-phase HPLC assay was carried out using a binary isocratic elution with a flow rate of 0.8 mL/min at a column temperature of 25˚C, a mobile phase of acetonitrile/0.1% acetic acid (1:3) and a detection wavelength of 254 nm. The

acquisition time for aloin injection was 15 minutes. Data were processed with LC-Solution Software. Solvents and water were filtered through a 0.45 µm nylon membrane with the aid of Buckner, enhanced by ILMVAC GmbH vacuum pump.

For the preparation of the calibration curves, standard stock solutions were prepared in methanol, filtered and appropriately diluted to obtain the desired concentrations. The calibration graphs were plotted as the linear regression of the peak areas versus concentrations. To determine the aloin contents, both gel and latex extracts (1 mg/mL in PBS) were analyzed using HPLC-UV. The amount of aloin was calculated from the calibration curve.

2.6. Statistical Analysis of Results

Quantitative data obtained from triplicate analyses were expressed as means ± standard deviations of replicates. To identify any significant differences between means, One-Way Analysis of Variance (ANOVA) was performed with Tukey post hoc test at $p < 0.05$. The analyses were performed using Graph Pad Prism for windows (v9.0, Graph Pad Software, California, USA).

3. Results

In this study, the extraction of aloin from *A. barbadensis* leaf latex and gel from three different regions of Kenya was performed using PBS to improve the stability of aloin. The aloin content was established in the presence of standard aloin to determine the optimal chromatographic conditions for its detection. **Figure 1** shows the calibration curve used in the quantification as the linear regression of the peak areas versus concentrations.

Figure 2 is the chromatogram obtained for the aloin standard and the gel and latex extracts. The peak for aloin standard was observed at 13.589 minutes. Aloin in the extracts was identified by comparing their retention times with that of the standard and spectral matching with the standard aloin's characteristic spectrum. To improve the identification of aloin in the extracts, a precise amount of each standard was co-injected with each extract for HPLC detection. These ones confirmed the retention time of aloin standard. For the extracts (latex), aloin from Kisumu, Elgeyo-Marakwet and Baringo Counties had retention times of 13.8, 13.7 and 13.6 minutes, respectively (**Figure 2**).

3.1. Method Validation

Linearity of the standard curves was estimated as the determination coefficient (r^2) from plots of the integrated peak heights versus the concentrations of the standard (**Figure 3**). All the standard curves produced throughout the course of the study appeared linear upon visual inspection. The coefficients of determination (r^2) were ≥99.9%, therefore considered linear and acceptable for quantifying aloin in the extracts. Linear regression does not give equal weight to the calibration points especially at low levels when a seven-point calibration curve is used but the r^2 for four-point calibration curve was ≥99.9%.

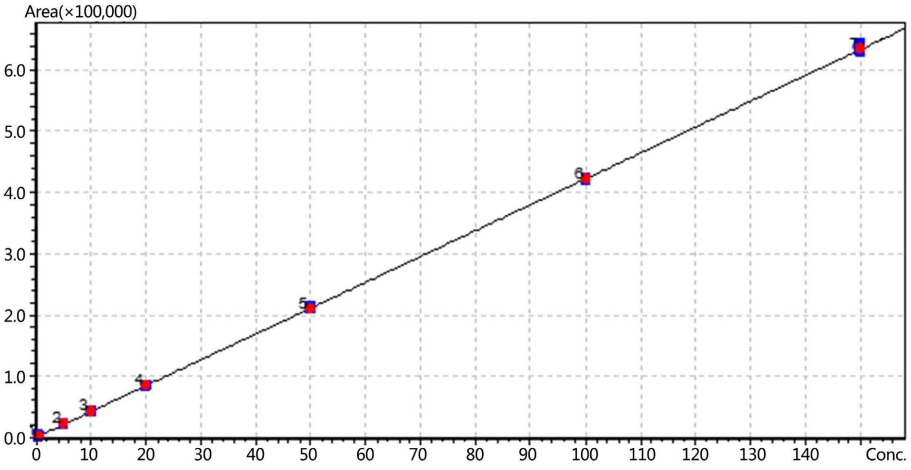

Figure 1. Calibration curve for quantification of aloin in A. barbadensis leaf latex and gel extracts.

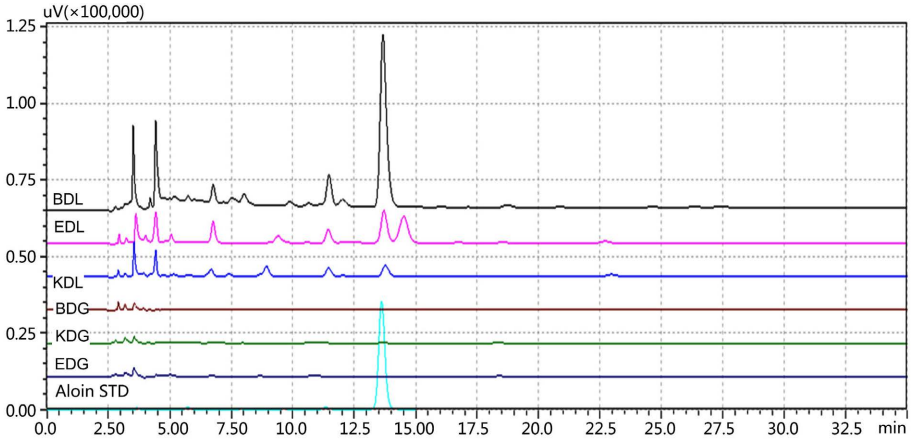

Figure 2. HPLC chromatograms of aloin standard (aloin STD) and aloin isolates from the sample extracts. **Legend:** BDL = Baringo dry latex, ELD = Elgeyo-Marakwet Dry Latex, KDL = Kisumu Dry Latex, BDG = Baringo Dry Gel, KDG = Kisumu Dry Gel, EDG = Elgeyo-Marakwet Dry Gel.

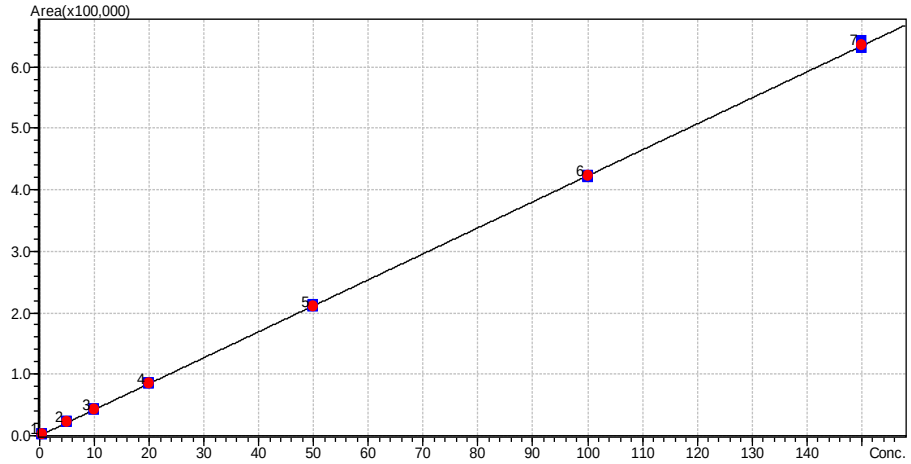

Figure 3. Determination coefficient (r^2) from plots of the integrated peak heights versus the concentrations of the standard.

Precision of the method was established in terms of the Relative Standard Deviation (RSD) which was found to be between 0.393% and 0.76% for repeatability. On the other hand, reproducibility of total aloin in the samples was established through analysis of the same samples for 3 days, and the RSD values for the samples were found to be less than 0.392 for the seven chromatograms.

3.2. TLC Profile and Aloin Content in Gel and Latex of *A. barbadensis* Leaves

The aloin contents of dry gel and latex samples were determined in different preparations of PBS extracts. Though the latex from the three regions were able to separate along with the aloin standard when dissolved in the same solvent system, the corresponding gels regions did not show any separations (Figure 4, Table 2).

As seen in Table 3, latex samples quantified had higher aloin amounts than the gels, with the highest content being 237.971 ± 5.281 mg aloin/g DW for dry latex from Elgeyo-Marakwet followed by those from Baringo (198.409 ± 2.000 mg aloin/g DW) and then Kisumu (40.760 ± 0.088 mg aloin/g DW). For gels, the aloin content followed the order Kisumu > Baringo > Elgeyo-Marakwet.

4. Discussion

In the study of Aloes, it is widely established that better extraction yields are obtained using methanol which in part explains its choice as the extractant for recovery of anthraquinones from this genus [20]. However, preceding authors

Table 2. Sampling location of *A. barbadensis* plants where leaves were sampled in Kenya.

Aloin standard	Kisumu dry gel	Kisumu dry latex	Elgeyo-Marakwet dry gel	Elgeyo-Marakwet dry latex	Baringo dry gel	Baringo dry latex
0.85	-	-	-	0.95	0.91	0.92
0.85	-	-	-	0.88	-	-
0.85	-	0.85	-	0.85	-	0.85
0.85	-	0.71	-	0.71	0.78	0.66
0.85	-	0.63	-	0.66	-	0.65
0.85	-	-	-	-	-	0.57
0.85	-	-	-	0.49	-	0.51
0.85	-	0.22	-	-	-	0.25
0.85	-	-	-	-	-	0.15

Table 3. Variability of aloin (mg/g DW) in samples of *A. barbadensis* leaves from Kenya.

Location	Dry latex	Dry Gel
Kisumu	40.760 ± 0.088	0.895 ± 0.004
Baringo	198.409 ± 2.000	0.363 ± 0.006
Elgeyo-Marakwet	237.971 ± 5.281	0.109 ± 0.004

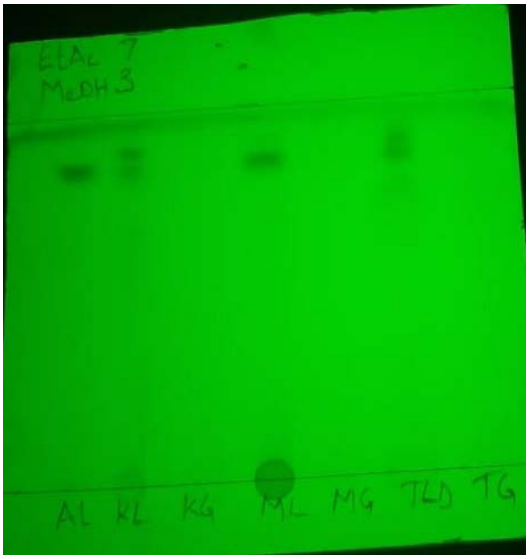

Figure 4. TLC fingerprints of the aloin standard and dry PBS extracts of *A. barbadensis* leaf latexes for ethyl acetate: methanol (7:3). **Legend**: AL = aloin standard, KL= Kisumu Latex, KG = Kisumu Gel, ML= Elgeyo-Marakwet Latex, MG = Elgeyo-Marakwet Gel, TLD = Baringo Latex Dry, TG = Baringo Gel.

have indicated that aloin content of methanolic extracts of *Aloe* tends to decrease gradually due to the formation of aloe-emodin, *i.e.*, poor stability [28]. This is particularly more pronounced at basic pH [21] [31]. Aloin is most stable at pH 3, and this explains the use of PBS at the same pH in this study instead of methanol [22] [26].

TLC profiling of the aloin isolates showed that the retention factor values were close to that of the aloin standard, confirming that they were pure aloin isolates. Upon quantification, latex extracts had higher aloin contents than the gels which is contrary to a report from Mexico [22] in which PBS extracts of *A. barbadensis* leaf gels contained higher quantities of aloin than the latex extracts. These differences may be explained by the differences in the climatic conditions of Kenya and Mexico, and the ages of the plants from which the leaves were harvested in the studies [21]. Similarly, the observed variations in the aloin content of the extracts from the different counties of Kenya could be due to variations in soil chemistry, topography and climate (growing conditions of the aloe plants) which are known to influence the distribution of phytochemicals in plant organs [5] [32] [33] [34]. Aloin makes up to 30% of the exudates from *Aloes* [21] [35]. Previous studies such as Lucini *et al.* [36] and Park *et al.* [19], reported aloin contents of 24.41 mg/g DW of gel and 1.14 ± 0.39 mg/g (for freeze-dried ethanolic extracts) which are close to some of the values obtained from the gels in this study. The paucity of data on the aloin content of dry latex and gel of *A. barbadensis* is in part explained by challenges associated with freeze drying fresh *Aloe* gel and latex samples, and the notion that fresh gel and latex have higher aloin content.

In the HPLC method validation, the correlation coefficients were found to be satisfactorily greater than 0.999, which is acceptable [22]. Precision expressed as repeatability and reproducibility had RSD values between 0.393% and 0.76%, respectively. In reference to previous reports, RSD values between 0.3% and 0.6% [24], 3.47% and 4.33% [17], 1.46% [37] and less than 5.0% [22] have been reported. The results of the current study, therefore, suggest that the method can be harnessed for the quantification of aloin from PBS extracts of *Aloe* species.

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Conflicts of Interest

The authors declare no conflicts of interest.

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