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Distribution of aloin-A in different leaves of *Aloe*

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ABSTRACT

Aloe (*Aloe barbadensis* Mill.) is one of the most important herbs used in medicinal and cosmetic preparations. Aloin-A is the major active compound in aloe exudates. It is present in the rind of leaves and is commercially used in several drug preparations. A study was conducted to find out the relationship between leaf age and quality parameters such as aloin-A content, weights of leaf exudates, fresh leaf, rind and gel with a view to find out optimum stage for harvesting of leaves. The aloin-A content was estimated by HPLC method and expressed as per cent on dry weight basis. Aloin yield and weight of exudates had very high and significant correlation (r = 0.983). Highest aloin-A content was recorded in the younger leaves (20.12% in 2nd leaf) which declined with leaf age. Weight of leaf exudates increased from 46.43 ± 5.41 to 460.88 ± 18.18 mg along with increase in leaf age. Maximum yield of the exudates (460.88 ± 18.18 mg) was obtained in the fully matured leaves (9-12 month-old). Hence, for obtaining the maximum yield of aloin-A, harvesting of leaves should be done when the leaves attain maturity, *i.e.* of about 9-12 month-old.

Key words: *Aloe barbadensis*, aloin-A, gel content, HPLC analysis.

INTRODUCTION

Aloe barbadensis Mill. (*A. vera* L.), a member of family Liliaceae, is a native of Africa and Mediterranean countries from where it had spread to the Asian countries including India. *Aloe* species have been in therapeutic uses since Roman times (Crosswhite and Crosswhite, 1; Morton, 7). Aloin and gel present in leaves are used for medicinal as well as cosmetic purposes. Aloins are mixture of anthraqiunone glycosides (Joshi, 3), which have purgative, analgesic, anti-inflammatory and anti-cancerous properties. Aloe gel is being extensively used in cosmetics preparations and in a number of skin tonics. The raw material of aloe is valued about US$ 65-80 million in global market (International Aloe Science Council, Inc. News, October 2004).

Cultivation of aloe has been started in recent years in Rajasthan, Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu where about 1,000 ha area is under its cultivation (Maiti, 5). Selection for high aloin yielding cultivars of *A. barbadensis* is initiated by different R& D organizations. Samantaray and Maiti (8) developed fast multiplication protocol of aloin rich lines in the species. To optimise the yield and profitability in aloe cultivation, basic information on the quality, aloin content, *etc*. in relation to leaf growth stages of the crop is essential. Hence, the present study was conducted to evaluate the gel and aloin content in leaves of different ages on a plant to help farmers/cultivators to plan the right stage of harvest of leaves.

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MATERIALS AND METHODS

Accession IC-283655 of *A. barbadensis*, which was grown at Experimental Farm DMAPR, Anand was used for the present study. Ten plants of about 18-month-old were selected. Average number of leaves per plant was twelve. The leaves were numbered downwards starting from the growing apex. The first four leaves were considered as young, next four as matured and last four as old leaves. The leaf samples were collected from the field and processed for aloin estimation and gel content.

For aloin-A estimation, individual leaves were cut at the base and exudates were collected separately in a beaker. The exudates were vacuum dried at 40°C for eight hours. Ten milligram of dried exudates was powdered, homogenised (Polytron PT 2100, Switzerland) and dissolved in 10 ml methanol. The solution was centrifuged at 8000 rpm and supernatant was filtered through 0.45 µm nylon syringe filter (Whatman, USA). The filtrate was used for HPLC analysis. Leaf gel was collected directly by scooping the gel from the leaf after collection of exudates. HPLC system (Shimadzu, Japan) equipped with LC-10AD pumps, SPD-10A UV-VIS detector and Aimil Chromatographic data station was used. HPLC column RP-18 (250 mm × 4.6 mm, 5 µm, LiChrospher, Merck) was used. UV-VIS detector was set at λ_{max} 254 nm for detection of aloin-A (Kawai *et al.*, 4). Standard aloin-A (barbaloin) was obtained from Sigma-Aldrich, USA. Solvents (methanol and water) used were of HPLC grade and obtained from Merck, India. Injection volume was 20 µl. Mobile phase

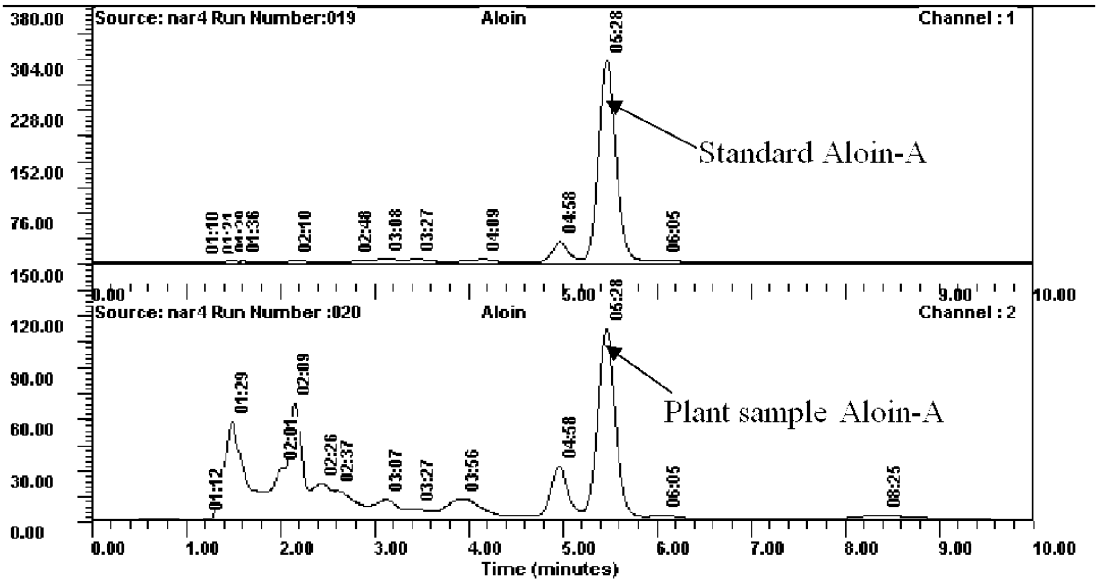

Fig. 1. HPLC chromatograph of standard aloin-A and extracted aloin-A from plant sample.

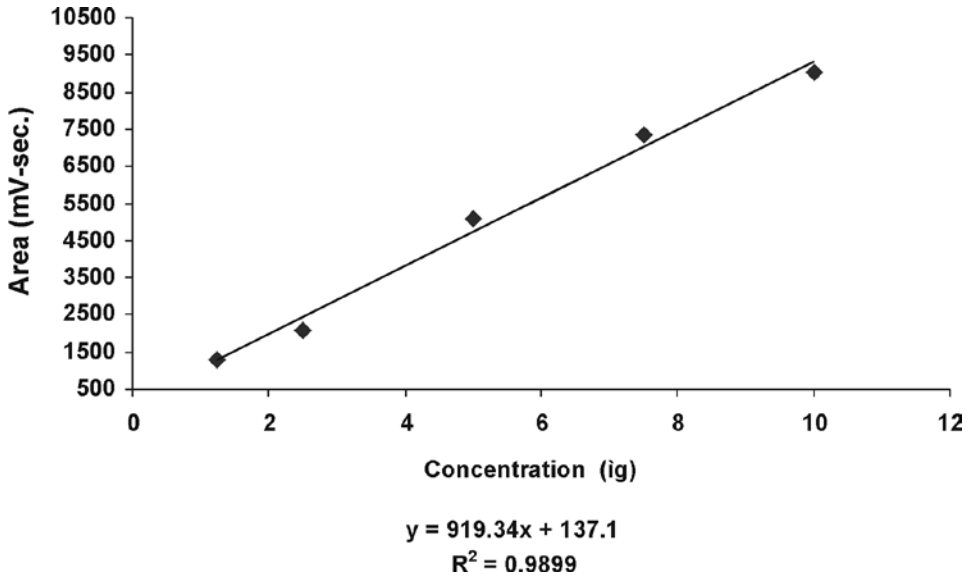

Fig. 2. Calibration curve for the determination of aloin-A by HPLC.

was a mixture of methanol and water (60:40) at a flow rate 0.8 ml min^{-1}. Under these conditions, the retention time of aloin-A was 5.28±0.05 min.

Calibration curve for aloin-A was constructed by injecting standard aloin-A solutions of different concentrations and subjecting the data to regression analysis. Aloin-A content and yield were calculated on the basis of dry weight of leaf exudates. Other observations recorded in the study were fresh gel weight, moisture content of gel, rind weight and weight of leaf exudates.The data were analyzed using MSTAT-C (Michigan State University, USA) statistical programme and standard error was calculated.

RESULTS AND DISCUSSION

HPLC conditions were optimized to get resolved peak of aloin-A from the other chemical constituents of aloe exudates (Fig. 1). As evident from calibration curve, there was good linearity (R^2 = 0.9899) between concentration and HPLC response (Fig. 2). The fresh leaf weight ranged from 23.94 ± 7.27 g per leaf (1st leaf) to 491.78 ± 19.15 g per leaf (10th leaf). Similarly

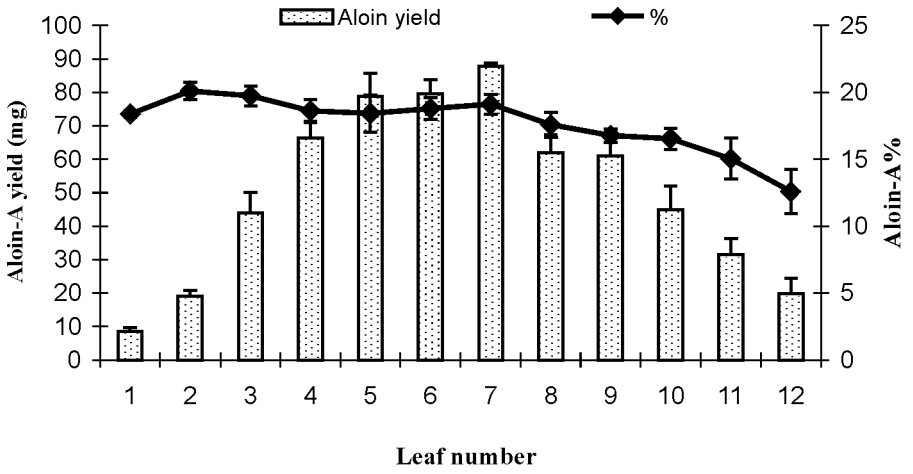

Fig. 3. Aloin percentage and yield in individual leaves of aloe plant.

Table 1. Quality parameters in relation to leaf age in *A. barbadensis*.

Group	Leaf No	Fresh leaf wt. (g)	Fresh gel wt. (g)	Fresh rind wt. (g)	Dry leaf exudates wt. (mg)	Aloin-A (%)
Younger	1	23.94 ± 7.27	11.85 ± 5.05	12.10 ± 2.55	46.43 ± 5.41	18.38
	2	59.08 ± 13.08	35.45 ± 7.98	23.63 ± 5.22	95.22 ± 11.07	20.12
	3	123.33 ± 24.30	79.54 ± 15.43	43.79 ± 8.88	220.80 ± 22.13	19.74
	4	207.58 ± 10.66	139.22 ± 9.34	68.37 ± 2.33	355.67 ± 10.21	18.61
Matured	5	274.33 ± 9.78	193.80 ± 7.69	80.53 ± 3.42	427.58 ± 17.54	18.43
	6	299.68 ± 30.82	216.48 ± 23.43	83.19 ± 7.59	422.66 ± 5.05	18.80
	7	360.22 ± 12.62	264.25 ± 10.78	95.97 ± 2.08	460.88 ± 18.18	19.10
	8	416.60 ± 27.56	317.78 ± 15.59	98.82 ± 12.19	352.38 ± 20.41	17.58
	9	460.49 ± 16.57	350.92 ± 8.65	109.57 ± 7.93	362.80 ± 24.34	16.76
	10	491.78 ± 19.15	378.10 ± 16.07	113.67 ± 3.66	301.93 ± 11.08	16.53
Older	11	453.30 ± 11.70	350.71 ± 9.54	102.58 ± 2.27	208.17 ± 23.36	15.05
	12	357.18 ± 33.81	271.47 ± 28.37	85.70 ± 5.72	154.06 ± 20.71	12.59
SEm±		13.87	10.46	4.70	16.04	-

fresh gel weight varied from 11.85 ± 5.05 g to 378.10 ± 16.07 g per leaf. The fresh leaf weight, gel weight and rind weight were minimum in 1st leaf and maximum in 10th leaf (Table 1). The value for these three parameters increased with age upto leaf maturity and then started decreasing. Almost similar trend was observed by Zhao *et al*. (9) in *Nicotiana tabacum*, where they found highest solanesol content in younger leaves which gradually decreased with maturity. Similarly in *Gymnema sylvestre*, the gymnemic acid content was found higher in younger leaves than matured and old leaves (Manohar *et al*., 6).

Weight of dry exudates of leaf increased with age upto maturity. It was least in first leaf and highest in matured 7th leaf and thereafter decreased considerably in the older leaves. The aloin-A yield was minimum (8.56 mg per leaf) in the 1st leaf (youngest leaf) and maximum 87.78 mg in the 7th leaf (matured leaf) but thereafter decreased upto 12th leaf and followed near normal curve distribution (Fig. 3). Similar variation and

Table 2. Correlation between aloin-A and other characters in *A. barbadensis*.

Parameter	Fresh gel wt.	Fresh rind wt.	Dry leaf exudate wt.	Aloin-A	Aloin yield
Fresh leaf wt.	0.999	0.973	0.530	-0.590	0.380
Fresh gel wt.		0.973	0.492	-0.614	0.339
Fresh rind wt.			0.663	-0.510	0.526
Dry leaf exudate wt.				0.182	0.983
Aloin-A					0.328
Aloin yield					

distribution for diosgenin content have been reported in *Costus speciosus* (Gupta *et al*., 2). Younger leaves had the highest percentage of aloin-A, which dropped gradually with increasing leaf age. Percentage of aloin-A was 12.59 in 12th leaf and 20.12 in 2nd leaf and it decreased with leaf maturity. The aloin content had strong negative correlation with fresh leaf weight (Table 2). Interestingly, the aloin-A content (expressed as per cent aloin-A per g dry weight) was constantly maintained in the young and matured age group. The content decreased further in the older leaves having the least aloin-A.

A characteristic pattern emerged for the distribution of aloin-A in the leaf groups (Fig. 3). The yield of aloin-A was distributed unevenly, the younger and old leaves had lower yield and the matured leaves had the higher yield (Table 1).The lowest yield was expected in youngest leaves, since weight of fresh leaf was low. The yield increase with the age of the leaf was also expected because of the active physiological growth during the period. Once the leaves attained full maturity, the growth and some physiological process may be stopped or reduced. Considering the distribution of aloin content and yield in different leaves of aloe in the present study, it is advisable to harvest the leaves when they reach maturity to obtain maximum yield of aloin-A. The harvesting of young leaves or over matured (old) leaves would lead to lower yield. The correlation between the different quantitative and qualitative characters in aloe is presented in Table 2. The aloin and exudates yields were significantly correlated (r = 0.983), indicating that the high aloin yield genotypes could be identified just by selecting high exudates yielding genotypes. Aloin yield had low and positive relation with all the other parameters like fresh leaf weight, gel weight, aloin percentage and gel moisture percentage and rind weight.

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