Geographical Variation in the Major Compounds of *Aloe ferox* Leaf Exudate

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Received: June 17, 1994; Revision accepted: August 27, 1994

Abstract

Geographical variation in fresh *Aloe ferox* leaf exudate of which the dried product is commercially known as Cape Aloes, was investigated throughout the natural distribution range of the species. The composition of the major compounds is remarkably invariable, with aloeresin A, aloesin, and aloin (both epimers A and B) contributing between 70 % and 97 % of total dry weight, in a ratio of approximately 4 : 3 : 2, respectively. Minor compounds are less evenly distributed, with aloinoside A and aloinoside B more frequent in the western parts of the distribution area and aloeresin C and 5-hydroxyaloin A generally present in small quantities throughout the distribution area. The aloin content of the exudate is clearly related to provenance but there are no distinct geographical discontinuities. The selection of high-yielding provenances, with total aloin levels above 25 %, is recommended for commercial cultivation.

Key words

Aloe ferox, Asphodelaceae, geographical variation, leaf exudate, Cape Aloes, aloin, anthrones, chromones.

Introduction

Leaf exudates from *Aloe* species are used to a great extent in traditional medicines, both in humans and livestock (1). The main source of African drug aloes is *A. ferox* Mill., a species restricted to South Africa (Fig. 1). The leaf juice is collected and dried by a traditional method to produce a dark brown solid substance known as bitter aloes or Cape Aloes. The larger part of the annual production is exported to Europe (mainly Italy, France, and Germany) (2), but substantial quantities are also marketed and used locally. The chemistry of *A. ferox* leaf exudate has been the subject of several studies and is relatively well known (3–9). The main purgative principle is the anthrone *C*-glucoside, aloin (= barbaloin), which occurs as two diastereoisomers (10, 11). The aloin content of the dried exudate is reported to vary between 8.5 and 30 % (12–14) but 18 % is the minimum requirement for the exported product as described in the monograph for *Aloe capensis* in the European Pharmacopoeia (15) and Cape Aloes in the British Pharmacopoeia (16).

Despite the importance of Cape Aloes as a South African export product, surprisingly little is known about the extent of variation in the major chemical compounds. In this paper, we report on a rigorous comparison of *Aloe ferox* leaf exudates throughout the natural distribution area. A better understanding of the geographical variation was needed in view of broad generalizations that have been made about the quality of the product, based on small numbers of drug samples. A further aim was to identify superior provenances for selection and commercial cultivation.

Materials and Methods

Plant material

Between two and seven plants from each of 37 natural populations of *A. ferox* were sampled, resulting in a total of 127 samples (Table 1). To deposit large numbers of herbarium specimens would have been unnessary, for the following reasons: 1, all plants were identified *in situ* by *Aloe* taxonomists (authors of this paper); 2, localities were carefully noted; 3, there are no species similar to *A. ferox* (chemically and morphologically) in the natural distribution area and sampling was done in the flowering season. The provenances are listed in Table 1 and their geographical distribution is shown in Figure 1. Seasonal variation was excluded by taking all the samples in a single month (July 1993). Juice was collected from the apex of the fifth leaf of each rosette, spread on filter paper, and air-dried within a few minutes. This method of sampling allows a rigorous comparison of the major compounds, particularly the levels of aloin in various geographical areas. Commercial samples of Cape Aloes from six different suppliers were pre-cleaned and analysed in the same way as the exudate samples. Duplicate samples of all the materials analysed are available on request.

Procedures

It has been shown (17) that HPLC analysis gives an accurate estimate of the aloin content of the crude extract. We confirmed earlier findings (13) that the relative position of leaves in *A. ferox* [unlike other species, see (17)] have little effect on the aloin content. Samples were dissolved in methanol and passed through C_{18} cartridges to remove substances of high retention time. These pre-cleaned samples were dissolved in a methanol-water (1 : 1) mixture and injected into the HPLC system. A Phenomenex IB-Sil column was used (C_{18} reverse phase, 5 μm particle

Planta Med. 61 (1995) 250–253

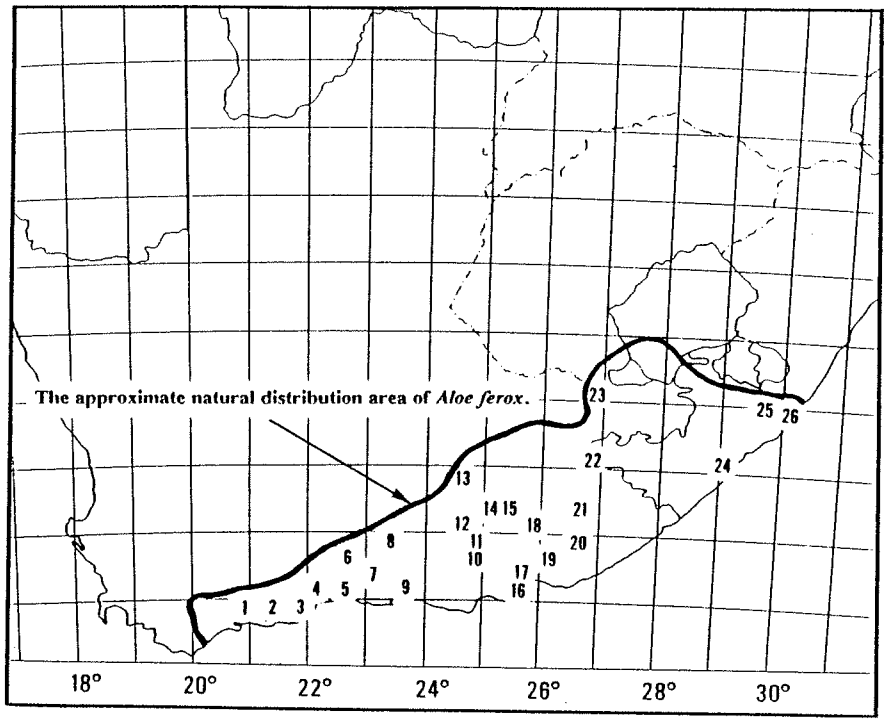

Fig. 1 The approximate geographical distribution of *Aloe ferox*, showing the localities (numbered 1 to 26, see Table **1**) where leaf exudate samples were collected.

Table 1 Localities of *Aloe ferox* where leaf exudate samples were collected. A total of 127 samples were taken from 37 localities.

Locality Number (see Fig. 1)	Locality (grid reference)	Number of Samples
1a	3.5 km west of Heidelberg (3420BB)	6
1b	3.0 km east of Heidelberg (3420BB)	3
2	3 km east of Riversdale (3421AB)	3
3a	Gouritz River, eastern bank (3421BB)	3
3b	2 km from Cooper to Herbertsdale (3421BB)	3
3c	12 km from Cooper to Herbertsdale (3421BB)	3
4a	Robinson Pass, top end (3322CC)	4
4b	Moerasrivier, near Robinson Pass (3322CC)	3
5	ca. 25 km from George to Uniondale (3322DC)	3
6	Meiringspoort, southern end (3322BC)	3
7	ca. 4 km from Uniondale to Oudtshoorn (3323CA)	3
8	Perdepoort, 16 km from Willowmore (3323AB)	6
9	18 km west of Joubertina (3323DC)	3
10	ca. 70 km south-east of Jansenville (3324BD)	3
11	Salt Pan's Nek, near Jansenville (3324BB)	3
12a	8 km south-east of Jansenville (3324DC)	3
12b	7 km from Jansenville to Pearston (3224DC)	3
13a	ca. 25 km from Graaff-Reinet to Middelburg (3224BA)	3
13b	Goliatskraal, ca. 20 km from Graaff-Reinet (3224BA)	6
13c	Vanryneveldspas Dam, at Graaff-Reinet (3224BA)	6
14	ca. 4 km from Pearston to Somerset East (3225CA)	3
15	Bruintjieshoogte, near Somerset East (3225CB)	3
16a	Aloes, near Port Elizabeth (3325DC)	7
16b	Coega Hill, near Port Elizabeth (3325DC)	3
17	Addo (3325DA)	6
18	Slagtersnek, near Cookhouse (3225DD)	3
19	45 km from Grahamstown to Port Elizabeth (3326AC)	3
20	Fort Brown (3326BA)	3
21	10 km from Seymore to Fort Beaufort (3226DA)	3
22	10 km south-east of Queenstown (3126DD)	3
23	14 km from Aliwal North to Jamestown (3026DD)	3
24a	Coffee Bay, Transkei (2931CC)	3
24b	4 km north of Coffee Bay (2931CC)	3
24c	Canzibe, 25 km from Coffee Bay to Umtata (2931CC)	2
25	Bizana, Transkei (2930DD)	2
26a	Umtamvuna River Mouth, Transkei side (3031AA)	2
26b	Umtamvuna River Mouth, Natal side (3031AA)	2

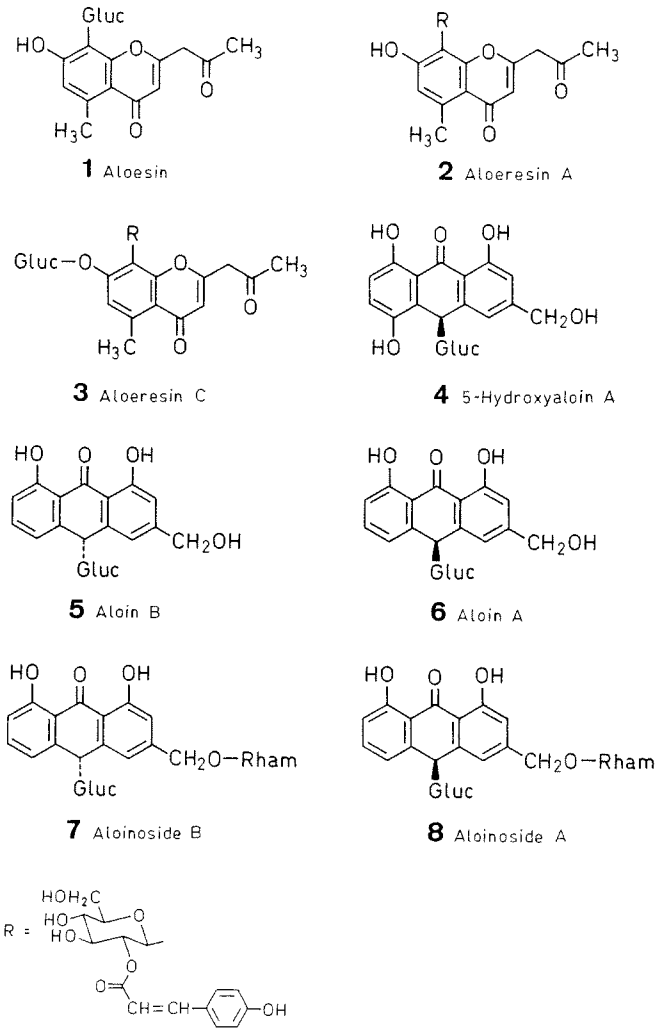

Fig. 2 Chemical structures of the major compounds in *Aloe ferox* leaf exudate.

Table 2 Composition of *Aloe ferox* leaf exudate in 37 natural populations (see Table **1**) and 10 commercial samples of Cape Aloes. Only one example of each provenance is included to show the range of variation found in all the samples.

Locality or sample	% Composition of the leaf exudate									
(see Table **1**)	aloesin (**1**)	aloeresin A (**2**)	aloeresin C (**3**)	5-OH-aloin A (**4**)	aloin B (**5**)	aloin A (**6**)	aloinoside B (**7**)	aloinoside A (**8**)	total % aloin (**5** + **6**)	% aloin (population mean)
Rt (minutes):	5.78	14.60	11.15	21.09	24.76	26.11	28.21	30.36		
Natural Populations:										
1a	23.3	32.6	4.4	3.3	11.5	12.0	2.2	2.1	23.5	24.3
1b	28.7	33.6	0.5	3.0	10.5	11.1	2.2	2.2	21.6	22.6
2	24.5	26.1	5.5	6.5	12.6	13.2	1.6	1.2	25.8	25.6
3a	21.4	28.4	4.0	3.5	15.0	16.2	1.3	1.3	31.2	29.0
3b	22.2	29.8	5.0	–	13.7	13.9	0.6	0.4	27.6	25.4
3c	22.5	24.2	4.9	1.7	13.4	15.0	0.1	0.4	28.4	23.8
4a	22.1	42.7	4.0	–	10.5	11.4	0.9	0.8	21.9	25.4
4b	29.0	38.8	0.6	0.9	10.2	10.9	1.0	0.9	21.1	20.7
5	29.6	35.0	0.3	4.1	11.0	10.8	0.4	0.4	21.8	22.3
6	26.3	37.7	0.6	1.1	13.2	14.6	–	–	27.8	26.3
7	27.2	43.8	1.0	3.9	9.5	10.8	–	–	20.3	20.4
8	23.1	41.9	1.6	tr	12.7	12.9	–	–	25.6	23.2
9	28.9	35.7	0.7	5.1	8.9	10.2	–	–	19.1	19.4
10	26.1	35.8	0.6	1.9	9.2	8.2	–	–	17.4	16.7
11	26.7	30.6	1.2	1.8	6.8	7.6	–	–	14.4	15.2
12a	24.3	33.7	3.5	1.9	8.0	8.7	–	–	16.7	17.8
12b	31.5	39.0	1.2	1.4	8.4	9.1	–	–	17.5	18.6
13a	25.4	30.4	5.5	1.9	11.7	13.0	0.7	0.3	24.7	26.2
13b	15.4	43.7	2.6	2.4	13.5	15.1	–	–	28.6	25.2
13c	25.0	27.4	3.3	1.7	11.2	11.1	0.6	0.2	22.3	20.6
14	21.7	32.4	0.8	2.1	14.5	16.1	tr	tr	30.6	26.1
15	23.7	31.5	3.6	–	12.4	12.3	tr	tr	24.7	24.5
16a	28.9	36.2	6.2	2.8	6.9	7.6	tr	tr	14.5	16.0
16b	20.2	44.1	tr	3.2	7.1	7.9	–	–	15.0	14.2
17	27.5	31.4	2.4	1.9	10.7	11.8	–	–	22.5	21.7
18	23.8	36.2	0.4	1.3	12.4	13.1	–	–	25.5	24.4
19	21.7	28.8	1.3	–	13.3	12.0	0.1	tr	25.3	21.4
20	25.5	39.0	0.8	–	7.6	7.8	–	–	15.4	14.6
21	19.6	30.8	1.1	–	11.3	10.5	–	–	21.8	22.8
22	24.4	34.2	1.7	4.3	10.7	10.4	–	–	21.1	21.0
23	27.0	33.5	1.1	1.6	13.9	15.4	–	–	29.3	29.1
24a	20.8	36.8	5.8	4.2	5.4	5.3	–	–	10.7	12.9
24b	31.7	44.3	4.7	tr	8.5	9.8	–	–	18.3	17.9
24c	25.3	37.3	5.1	3.3	4.7	5.0	–	–	9.7	16.6
25	30.2	38.0	0.5	6.1	7.8	8.6	0.2	0.3	16.4	17.7
26a	30.9	52.3	tr	3.7	4.9	5.2	–	–	10.1	11.6
26b	30.9	51.1	tr	4.0	5.1	5.1	–	–	10.2	10.2
Commercial Samples: (1 to 6 are examples of local retail samples; 7 is a typical example of a retention sample from a large manufacturer of pharmaceutical products and 8 to 10 were taken from bulk export samples)										
number-distributor-origin										
1-A-unknown	37.4	44.6	2.4	0.7	5.2	5.2	–	–	10.4	
2-A-unknown	24.1	35.6	3.3	2.6	8.8	8.7	–	–	17.5	
3-A-unknown	38.2	42.2	2.4	0.7	4.6	4.6	–	–	9.2	
4-B-unknown	31.6	41.2	4.1	–	6.4	7.0	–	–	13.4	
5-B-unknown	23.5	33.9	5.9	0.4	7.4	7.9	–	–	15.3	
6-C-unknown	32.7	40.8	3.1	0.8	6.5	6.4	–	–	12.9	
7-D-Port Elizabeth	27.8	37.7	2.3	–	8.6	8.0	–	–	16.6	
8-E-Albertinia	26.2	31.6	2.3	3.3	11.8	11.8	2.9	2.7	23.6	
9-F-Gouritz Riv.	23.9	33.0	2.5	3.5	11.5	11.4	2.5	2.1	22.9	
10-F-Herbertsdale	24.4	35.2	1.8	3.8	12.2	12.1	2.1	1.9	24.3	

size, 250 mm × 4.6 mm internal diameter; flow rate 1 ml min^{-1}; 20 µl sample loop). The solvent system comprised a 30% to 60 % linear gradient of methanol in water over 25 min, 3 min isocratic, 100% in 2 min, 4 min isocratic. For general screening this system was changed as follows: 40% to 80% in 12 min, 80% to 100% in 1 min, isocratic for 4 min. Detection was by diode array detector, using two channels (A set at 275 ± 70 nm; B set at 365 ± 40 nm). The samples were also analysed by TLC on silica gel (Merck) plates using ethyl acetate-methanol-water (100 : 16.5 : 13.5) as eluent. Identification of the compounds was achieved by direct TLC and HPLC comparisons (R-values, visibility/colour under UV 254 and 366 nm, retention times, UV/VIS spectra) with reference samples isolated or obtained from various sources as described below. Aloesin and aloeresins A, C and D were kindly sent to us by Prof. G. Speranza. The identity of aloinosides A and B was confirmed by direct comparison with authentic reference samples isolated from *A. africana* Mill., where these rhamnosides occur as major constitutents (4, 5). The major compound of *A. broomii* Schonl. was isolated by preparative HPLC and it subsequently proved to be identical (TLC, HPLC and NMR) to 5-hydroxyaloin A reported from the same source (8).

Results and Discussion

The composition of major and some minor constituents (Fig. 2) in one exudate sample from each of the 37 localities of *A. ferox* is shown in Table 2, together with a comparison of ten commercial samples. Aloesin, aloeresin A (a coumaric acid ester of aloesin), and the two diastereomers of aloin (aloin A and B) are the only major compounds, and are present in a ratio of ca. 2.5 : 3.5 : 1 : 1, respectively. The aloin content of juice samples varied between 9.5 % and 31.2 %, with an overall mean of 21.1 %. Similar results were obtained for commercial samples. In samples 1 to 7, the aloinosides A and B (rhamnosides of aloin A and B) were totally absent, while small amounts were present in samples 8 to 10 from the Mossel Bay region. Small quantities of aloeresin C and 5-hydroxyaloin A (usually around 3 % but rarely up to 15 %) are present in most of the samples. The aloinosides occur less frequently, in concentrations of up to 3 % each.

The overall composition of major compounds in *A. ferox* leaf exudate is remarkably invariable, especially when the morphological variation and wide natural distribution area of this species are considered. In contrast, the morphologically similar and equally widespread *A. marlothii* Berger shows tremendous variation in its anthracene compounds (6, 8). In *A. ferox*, aloesin, aloeresin A, and aloin contribute 70 % to 97 % of the total dry weight of leaf exudate (the leaf juice as well as the dried commercial product). This combination appears to be a useful chemotaxonomic character to distinguish *A. ferox* from related species. The method used to dry the juice seems to have a limited effect on the composition of major compounds and our results support a recent conclusion (18) that the major compounds of the dried juice are remarkably stable. There are some qualitative differences in minor compounds (such as the irregular occurrence of aloinoside B, aloinoside A, and 5-hydroxyaloin A) but the chemical discontinuity between two chemovars (3) – Type A from Mossel Bay and Type B from Port Elizabeth – is not closely correlated with geographical patterns. The two aloinosides (previously thought to be limited to Type A) are mainly found in the western populations but they also occur sporadically elsewhere, as was shown recently (19). Nevertheless, these minor compounds are useful in determining the origin of commercial samples, especially when the most likely source areas are known. Our results confirm the report (7) that aloesin and aloeresin A, as well as the two isomers of aloin, are the major constituents of Cape Aloes, and that smaller amounts of 5-hydroxyaloin A are usually present (8, 19). The latter compound and aloeresin C may be totally absent in some individuals (see Table 2). Several minor compounds have been isolated from commercial samples but some of these may be artefacts or contaminants. The phenylpyrone glucoside aloenin B, for example, was isolated from Kenya Aloes (20) (said to be from cultivated hybrids of *A. ferox*) but aloenin B was not detected in any of our samples.

The proportion of aloin relative to other compounds varies considerably, both within and between populations. It is important to note that our figures refer to the aloin concentration and not to total anthrone content (15, 16). HPLC analysis gives an accurate estimate of the aloin level (17) but only a conservative estimate of total anthrones, since aloe-emodin, 5-hydroxyaloin A, and the two aloinosides are not included in the calculation. There are no distinct geographical trends but aloin levels are clearly related to provenance. Aloin concentrations are consistently above 20 % in the western part of the distribution area, and sporadically also high (up to 30 %) in the eastern parts. The overall mean level of aloin is about 21 %, and commercial yields can no doubt be improved by selecting suitable localities for harvesting. The suggestion that *A. ferox* is not the best choice for commercial utilization (17) did not take into account the method of tapping (large leaves with thorns along the margins are required to produce the circular stacks) and also overlooked the much higher levels of aloin in natural populations compared to cultivated plants. Cape Aloes is an important source of income in rural areas and the way in which this labour-intensive industry develops in future has significant socio-economic implications. Commercial cultivation has already started on a small scale (mainly as enrichment plantings) and it may be worthwhile to select high-yielding provenances for this purpose.

Acknowledgements

This work forms part of a larger project on the chemosystematics of the family Asphodelaceae. We thank Pieter J. D. Winter and Alvaro M. Viljoen for help with the analyses and Prof. G. Speranza for supplying us with authentic samples. Funding from the Foundation for Research Development and South African Druggists Limited is gratefully acknowledged.

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