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High-performance liquid chromatographic determination of phenolic compounds in *Aloe* species

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Abstract

A procedure has been developed for determination of aloesin, 2′-*O*-feruloylaloesin, aloeresin A, barbaloin, isobarbaloin, aloenin, aloe-emodin, 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, isoaloeresin D and aloeresin E which are phenolic constituents of aloe. Aloe or commercial aloin was extracted with methanol multiple times, centrifuged and then filtered. Filtrates were analyzed by a reversed-phase high-performance liquid chromatography employing UV–Vis detection (290 nm). The samples were separated with a Wakosil-II 5C18 HG column by linear gradient elution using water–acetonitrile (88:12 to 54:46) as the mobile phase at a flow-rate of 1.0 ml/min. The detection limits of these compounds were 0.04–0.35 ng per injection (5 μl) and linearity of response existed. Very satisfactory and reproducible results were obtained within 38 min for simultaneous determination of these compounds. This method was applied to determine these compounds in *Aloe barbadensis* Miller, *A. arborescens* Miller *var. natalensis* Berger, *A. vera var. chinensis* Berger, *A. marlothii* Berger and *A. striata* Haw. Two commercial aloins were also analyzed.

Keywords: Aloe; Phenolic compounds; Aloesin; Aloeresin A; Barbaloin; Isobarbaloin; Isoaloeresin D; 2′-*O*-Feruloylaloesin; 8-*C*-Glucosyl-7-*O*-methyl-(*S*)-aloesol; Aloeresin E; Aloenin; Aloe-emodin

1. Introduction

Aloe genus (Liliaceae) constitutes about 600 species and these are known to occur mainly in Africa. Aloe is an important plant and is widely used as folk medicine. Two products are obtained from aloe leaves, both of which have been medicinally used for centuries. The fraction called gel of parenchyma cells, which is colorless and tasteless, has been used particularly for treatment of skin diseases [1]. In addition to a large amount of water this gel mainly contains polysaccharides. The yellow exudate from the inner epidermal cell layers is well known for its purgative activity [2], and phenolic compounds are abundantly contained in it. Purgative principles from aloe have been identified as an anthrone-*C*-glucosyl, barbaloin (aloin A) and homonataloin. In Japan, *A. arborescens* Miller *var. natalensis* Berger is used as a folk remedy, and *A. barbadensis* Miller (*Aloe vera*) attracts much attention as a health food. Aloesin (formerly named aloeresin B) barbaloin and the related compounds were isolated from the leaves of many *Aloe* species, and the antimicrobial activity and cathartic effects were confirmed.

Several papers on high-performance liquid chromatographic (HPLC) determination of barbaloin were reported [3–7], but only few describe the

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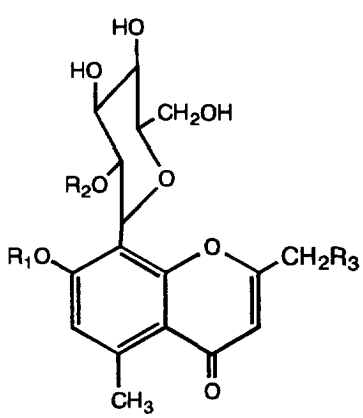
simultaneous determination of anthrone and chromone constituents [8,9]. Aloin, which is a commercial product made of aloe resin, contains barbaloin as a major constituent and is used instead of crude barbaloin. So, we developed a HPLC method for the simultaneous determination of several prominent constituents in *A. arborescens* Miller *var. natalensis* Berger, *A. barbadensis* Miller and commercial aloin. By this method the sample extracted with methanol can be analyzed without any pretreatment after filtration with a membrane filter.

The present study is aimed at the identification of ten compounds the formulas of which are given in Fig. 1. Aloesin, barbaloin and isobarbaloin (aloin B) are widely distributed over the *Aloe* genus plant. Aloenin and 2′-*O*-feruloylaloesin are found in *A. arborescens* Miller *var. natalensis*. Aloe-emodin is formed by air oxidation of barbaloin. Aloeresin A (*p*-coumaric acid ester of aloesin) was isolated from a commercial aloin. 8-*C*-Glucosyl-7-*O*-methyl-(*S*)-aloesol, isoaloeresin D and aloeresin E are the novel compounds which we found in *Aloe barbadensis* Miller [10]. In this report, we describe an application of this HPLC analysis for *Aloe* genus plants and commercial aloins.

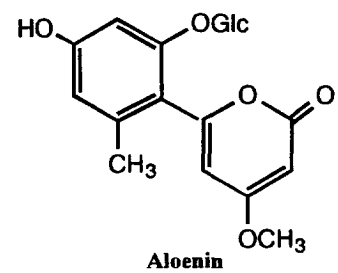
2. Experimental

2.1. Materials

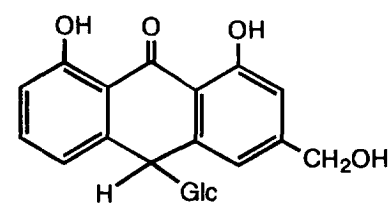
Specimens of *A. arborescens* Miller *var. natalensis* Berger, *A. barbadensis* Miller, *A. vera var. chinensis* Berger, *A. marlothii* Berger and *A. striata* Haw were available for inspection at the Herbal Garden, Fukuyama University. Dried leaf powder of *A. barbadensis* Miller and of *A. arborescens* Miller *var. natalensis* Berger was offered from Aloecorp (TX, USA) and Aloe Seiyaku (Shizuoka, Japan), respectively. Aloins were supplied by Sigma (St.



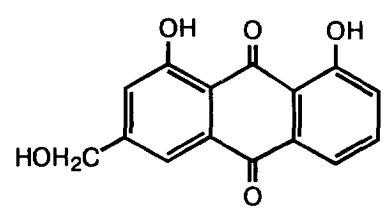
	R_1	R_2	R_3
Aloesin	H	H	$COCH_3$
8-*C*-Glucosyl-7-*O*-methyl-(*S*)-aloesol	CH_3	H	$CH(OH)CH_3$
Aloeresin A	H	*p*-coumaroyl	$COCH_3$
Isoaloeresin D	CH_3	*p*-coumaroyl	$CH(OH)CH_3$
2′-*O*-Feruloylaloesin	H	feruloyl	$COCH_3$
Aloeresin E	CH_3	cinnamoyl	$CH(OH)CH_3$



Aloenin



Isobarbaloin (10*S*)
Barbaloin (10*R*)



Aloe-emodin

Fig. 1. Structures.

Louis, MO, USA) and Nacalai Tesque (Kyoto, Japan). Reagent-grade chemicals and high-purity solvents were used except when specified otherwise. Ultrapure distilled water with a resistivity greater than 18 MΩ were prepared with deionized-distilled water. Acetonitrile was of HPLC grade and other solvents and chemicals were purchased from Wako (Osaka, Japan). Membrane filters (SJLH L04 NS, 0.45 μm pore size) were purchased from Nihon Millipore (Tokyo, Japan).

Barbaloin and aloe-emodin were purchased from Wako. Aloenin, 2′-*O*-feruloylaloesin and isobarbaloin were used as the authentic samples. Aloeresin A was prepared from a commercial aloin which was purchased from Nacalai Tesque. The commercial aloin (20 g) was subjected to MCI-gel CHP 20 P column chromatography (2200×600 mm I.D., 75–150 μm, Tokyo, Japan) using a stepwise gradient elution with water–methanol. The 30% methanol eluate (7.6 g) was subjected to repeated chromatography over a Sephadex LH-20 column (650×30 mm I.D., 25–100 μm, Pharmacia, Uppsala, Sweden) with methanol, 75% methanol and 50% methanol to give aloeresin A (2.4 g). The identification was done by comparison of the spectral data with those in the literature [11]. The other compounds were prepared from *A. barbadensis* Miller as described by Okamura [10].

2.2. Preparation of sample solutions

Samples (2–9 g) in methanol (20 ml) were homogenized with a Physcotron (Nition, Tokyo, Japan) while being cooled in ice and the mixture was centrifuged (1500 *g*) for 10 min. The methanol supernatant was then transferred to a test-tube, the residue was sonicated in a Branson ultrasonic bath (Shelton, CT, USA) for 10 min with methanol (10 ml) and centrifuged. The residue was extracted two more times in the same way. The extracts were combined and filtered, and then a portion was injected onto the HPLC column. When the content of desired constituents was low, solvent was blown off under a stream of nitrogen, and the residue was taken up in a proper amount of methanol and was used for HPLC analysis after filtration. Aloin was dissolved in methanol and after membrane filtration used for HPLC.

2.3. Instrumentation and HPLC analysis

The HPLC system consisted of two Tosoh (Tokyo, Japan) CCPD pumps equipped with a Tosoh CCP controller connected to a dynamic mixer, a Tosoh SD-8012 and a Tosoh UV-8020 UV–Vis detector set at 290 nm. Samples were injected with a Model 7125 (Rheodyne, Cotati, CA, USA) sample valve equipped with a 5-μl loop. More than 20 μl of methanolic solutions were injected. Peak areas were calculated with a SIC Chromatocorder-12 integrator (Tokyo, Japan). Separations were carried out with a Wako Wakosil-II 5C18 HG reversed-phase column (particle size of the packing 5 μm, 150×4.6 mm I.D.).

The mobile phase was a linear gradient of water–acetonitrile (0 min, 88:12; 19 min, 77:23; 24 min, 72:28; 39 min, 54:46) and degassed with the ultrasonic bath prior to use. A re-equilibration period of 15 min was used between individual runs. Chromatography was performed at 45 °C with a flow-rate of 1.0 ml/min. The identification and the purity of the chromatographic peaks were estimated using a Model 990J photodiode-array detector (Waters, Milford, MA, USA).

2.4. Solutions for recovery study

Recoveries of aloesin, 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, aloenin, aloeresin A, isobarbaloin, isoaloeresin D, barbaloin, 2′-*O*-feruloylaloesin, aloeresin E and aloe-emodin added to extracts of fresh inner epidermal tissues of *A. barbadensis* Miller and *A. arborescens* Miller *var. natalensis* Berger were measured by comparing three sets of chromatograms: standard solution, aloe extracts and spiked aloe extracts. Standards were prepared by diluting measured volumes of working standards with methanol to a known final volume. An appropriate amount of fresh inner epidermal tissues of *A. barbadensis* Miller and *A. arborescens* Miller *var. natalensis* Berger were weighed and extracted as previously described. Aloe extract was divided into four portions of one control solution and three spiked solutions. Aloe extract as control was brought to the same final volume. Spiked aloe extracts were prepared by adding measured volumes of working standards to aloe extracts, and brought to the same

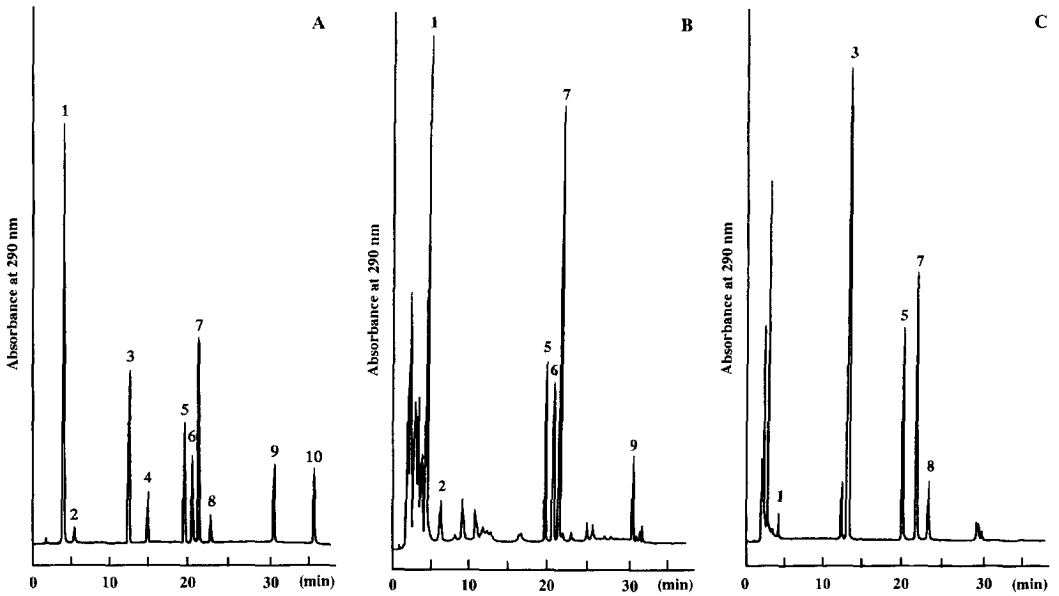


Fig. 2. Chromatograms of (A) standard mixture; (B) dried leaf powder of *A. barbadensis* Miller; (C) dried leaf powder of *A. arborescens* Miller *var. natalensis* Berger. Peaks (μg/ml: concentration of standard): 1=aloesin (103.2); 2=8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (10.2); 3=aloenin (60.4); 4=aloeresin A (21.4); 5=isobarbaloin (55.4); 6=isoaloeresin D (17.0); 7=barbaloin (67.4); 8=2'-*O*-feruloylaloesin (17.2); 9=aloeresin E (15.0); 10=aloe-emodin (19.6).

final volume. All samples were filtered through membrane filter and were injected for HPLC analysis to calculate the recovery.

3. Results and discussion

Separation was successful with a gradient elution of water–acetonitrile in less than 38 min (Fig. 2A). Absorption maxima of aloesin and barbaloin related compounds were observed around 250–295 nm on the UV spectra and UV detection was available at 290 nm in this paper. The retention times (and capacity factors, k') were: 3.94 (aloesin, $k'=1.35$); 6.93 (8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, $k'=3.12$); 12.37 (aloenin, $k'=6.56$); 14.58 (aloeresin A, $k'=7.68$); 19.27 (isobarbaloin, $k'=10.47$); 20.43 (isoaloeresin D, $k'=11.41$); 21.08 (barbaloin, $k'=$

Table 1
Within-day and day-to-day relative standard deviations (R.S.D.) for standard samples

Standard	Concentration (μg/ml)	R.S.D. (%)	
		Within-day[a]	Day-to-day[b]
Aloesin	50.2	0.88	0.53
8-*C*-Glucosyl-7-*O*- methyl-(*S*)-aloesol	113.2	0.46	2.80
Aloenin	44.4	0.77	0.76
Aloeresin A	34.1	2.23	2.00
Isobarbaloin	37.5	0.98	1.61
Isoaloeresin D	45.9	1.16	1.44
Barbaloin	56.3	1.46	0.95
2'-*O*-Feruloylaloesin	40.9	1.31	3.83
Aloeresin E	69.2	0.34	0.52
Aloe-emodin	48.8	0.79	1.18

[a] Within-day precision tested at five times in one day.
[b] Day-to-day precision tested on five different days.

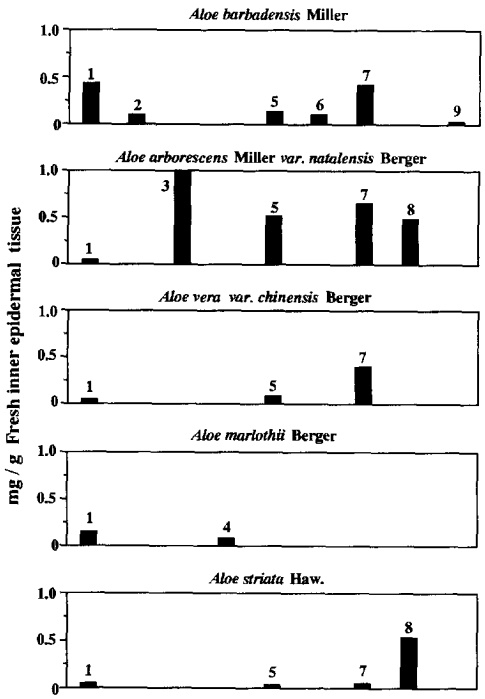


Fig. 3. Content of phenolic compounds in methanolic extracts of fresh inner epidermal layer from five *Aloe* species.

11.55); 22.64 (2′-*O*-feruloylaloesin, $k'=12.48$); 30.90 (aloeresin E, $k'=17.43$) and 35.67 min (aloe-emodin, $k'=20.23$). The purity of standard materials calculated from the peak area in the proposed method was ≥98%.

The detection limits (signal-to-noise ratio=3) were 0.06, 0.35, 0.13, 0.05, 0.09, 0.11, 0.07, 0.12, 0.04 and 0.06 ng per injection, respectively. The results of the precision of both the within-day assay and the day-to-day assay for standards are shown in Table 1. The calibration graphs for aloesin, 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, aloenin, aloeresin A, isobarbaloin, isoaloeresin D, barbaloin, 2′-*O*-feruloylaloesin, aloeresin E and aloe-emodin were linear within the range (0.05–850 μg/ml) where analyses are done.

In Fig. 2B and Fig. 2C typical chromatograms of *A. barbadensis* Miller and *A. arborescens* Miller *var. natalensis* Berger extracts are shown. These peaks, equivalent to standards, were shown to be satisfactorily separated under this condition. These peaks were identified with the retention time and the UV spectrum obtained by photodiode-array detection. This method can separate isoaloeresin D and aloeresin D (shorter retention time, $k'=10.73$) which are epimers at C-10 (data not shown).

The average recoveries of standards spiked into extracts of fresh inner epidermal tissues of *A. barbadensis* Miller and *A. arborescens* Miller *var. natalensis* Berger were 97% aloesin, 100% 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol, 101% aloenin, 104% aloeresin A, 101% isobarbaloin, 104% isoaloeresin D, 102% barbaloin, 92% 2′-*O*-feruloylaloesin, 99% aloeresin E and 95% aloe-emodin, for duplicated analyses.

The quantitative results in fresh inner epidermal

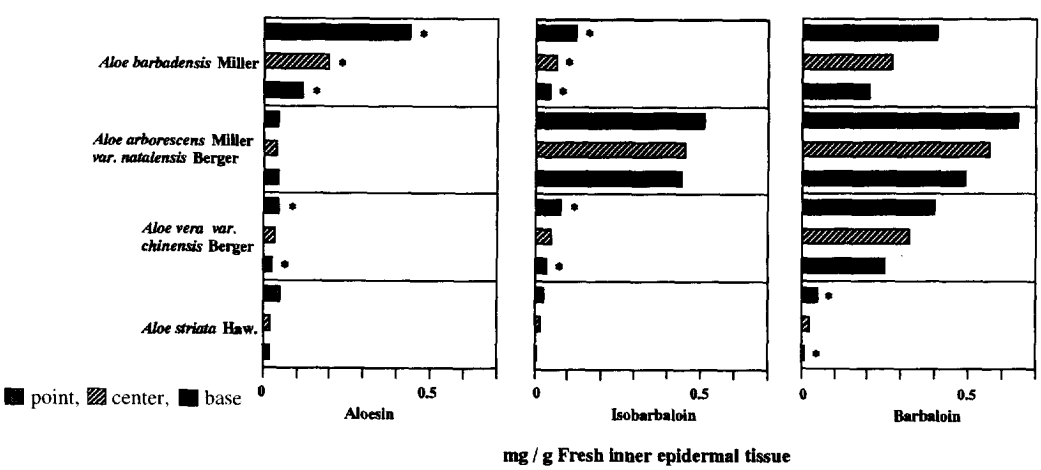


Fig. 4. Content of aloesin, isobarbaloin and barbaloin in fresh inner epidermal layer of different parts from each plant. The results were statistically evaluated by one-way repeated-measures ANOVA. *: $P<0.05$.

tissues of *A. barbadensis* Miller, *A. arborescens* Miller *var. natalensis* Berger, *A. vera var. chinensis* Berger, *A. marlothii* Berger and *A. striata* Haw are shown in Fig. 3. Aloesin, isobarbaloin and barbaloin were confirmed by their retention time and closely matched spectrum as major components except *A. marlothii* Berger. Aloenin [1.27 mg/g fresh tissue (f.t.)] occurred as a characteristic compound in *A. arborescens* Miller *var. natalensis* Berger. 8-*C*-Glucosyl-7-*O*-methyl-(*S*)-aloesol (0.09 mg/g f.t.), isoaloeresin D (0.10 mg/g f.t.) and aloeresin E (0.03 mg/g f.t.), which had (*S*)-configuration in C-10, were specifically recognized only in *A. barbadensis* Miller, while the corresponding diastereomer which had (*R*)-configuration was not found. In contrast to the widely distributed aloesin, barbaloin and isobarbaloin, these compounds may be usable as chemotaxonomic markers. In *A. striata* Haw, 2′-*O*-feruloylaloesin (0.53 mg/g f.t.) was the phenolic component found most abundantly. Rauwald and Beil [12] identified the existence of barbaloin and isobarbaloin by thin layer chromatography and HPLC in *A. marlothii* Berger. In our experiment, the peaks regarded as homonataloin A and B, a diastereomeric pair (longer retention time than barbaloin and isobarbaloin as a diastereomeric pair), existed with aloesin (0.14 mg/g f.t.) and aloeresin A (0.07 mg/g f.t.), whereas we were not able to find any barbaloin and isobarbaloin in *A. marlothii* Berger. No peak of aloe-emodin was detected in this study.

Reynolds et al. found that the highest concentrations of barbaloin [7] and homonataloin [13] were found in exudates from young mature leaves just below the apex and the level decreased in older leaves towards the base of the plant. Inner epidermal tissue of *A. barbadensis* Miller, *A. arborescens* Miller *var. natalensis* Berger., *A. vera var. chinensis* and *A. striata* Haw was divided into a point, a center and a base part, and the content of aloesin, barbaloin and isobarbaloin was compared for each part. As shown in Fig. 4, the content of these compounds increases clearly in the order: base, center and point. The content of these phenolic compounds relative to the part of the leaf decreases with age in aloe.

Gel, inner epidermal layer of leaf, terminal bud and root were analyzed to examine the distribution of aloesin, glucosyl-7-*O*-methyl-(*S*)-aloesol, isobarbaloin, isoaloeresin D barbaloin and aloeresin E in *A. barbadensis* Miller. The content per fresh weight of inner epidermal layer was 1.25–3 higher times than those of gel. In the root, only aloesin (0.01 mg/g f.t.) was identified.

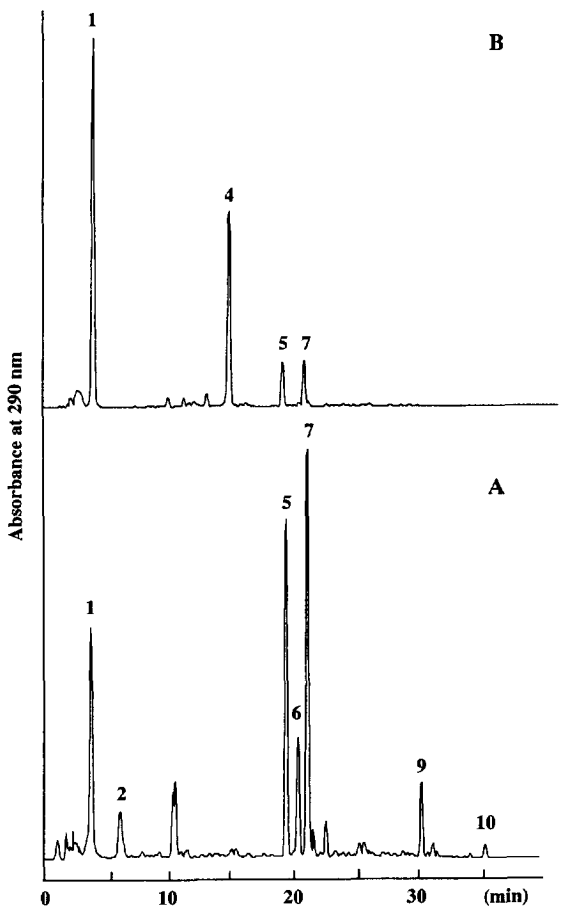


Fig. 5. Chromatograms of commercial aloins. Peaks : 1=aloesin; 2=8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol; 3=aloenin; 4=aloeresin A; 5=isobarbaloin; 6=isoaloeresin D; 7=barbaloin ; 8=2′-*O*-feruloylaloesin; 9=aloeresin E; 10=aloe-emodin.

Commercial aloin is utilized as a source of crude barbaloin, but is extremely different to manufacture. In the aloin products from Sigma, barbaloin, isobarbaloin and aloesin were found at 22.0%, 19.2% and 9.3%, respectively, and 8-*C*-glucosyl-7-*O*-methyl-(*S*)-aloesol (4.6%), aloenin (3.4%), isoaloeresin D (1.7%), aloeresin E (4.6%) and aloe-emodin (2.5%) were detected as shown in Fig. 5A. This distinctive chromatographic pattern resembles

that of *A. barbadensis* Miller. The aloin of Sigma was made of Curaçao Aloes (drug aloes). The dried latex of the leaves of *A. barbadensis* Miller has been known as a commercial source of Curaçao Aloe [14]. In the aloin by Nacalai Tesque there was a higher proportion of aloesin (43.7%) and aloeresin A (37.0%), and a lower proportion of barbaloin (8.3%) and isobarbaloin (8.1%) as shown in Fig. 5B.

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