In vitro activity of *Aloe* extracts against *Plasmodium falciparum*

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The antiplasmodial activity and toxicity data of 34 *Aloe* species and their main constituents were determined. A number of the methanol extracts possessed antimalarial activity where 50% of the parasite growth was inhibited (IC$_{50}$ value) by 32 to 77µg ml^{-1} of the extract. The chemical common to the most active species is the anthrone *C*-glucoside homonataloin which inhibited the chloroquine-resistant *Plasmodium falciparum* strain with an IC$_{50}$ value of 13.46 ± 1.36µg ml^{-1}. Homonataloin was a more potent inhibitor of parasite growth than aloin (IC$_{50}$ value of 107.20 ± 4.14µg ml^{-1}). The *Aloe* extracts tested did not exhibit any toxicity towards transformed human kidney epithelium cells at the concentrations (0.5–50µg ml^{-1}) used in the antimalarial assay.

Introduction

Aloes have been used since antiquity for various phytotherapeutic purposes. A wide range of cosmetic and therapeutic benefits have been ascribed to *Aloe* products derived from both the gel and the exudate. The most popular medicinal uses include the wound healing properties of *Aloe vera* and the cathartic effect of *Aloe* anthraquinones. In addition to a wide range of commercial products derived from aloes, many species form an integral part of African traditional healing and scientific evidence is continuously documented in support of the numerous traditional uses. Aloe-emodin possesses antiviral activities against poliovirus type 3, herpes simplex virus type 1 and 2, varicella-zoster virus, pseudorabies virus and the influenza virus (Sydiskis *et al*. 1991, Semple *et al*. 2001). Antiprotozoal activity of aloe-emodin against *Trypanosoma brucei brucei* (IC$_{50}$ value of 14 µM) has been described (Camacho *et al*. 2000); while Agarwal *et al*. (2000) reported the antifungal properties of aloe-emodin against *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Aspergillus fumigatus* (MIC range of 25–250µg ml^{-1}). Aloes with antiparasitic activity include the aqueous extract of *A. barbadensis* against *Trichomonas vaginalis* (Rojas *et al*. 1995).

Although aloes are not commonly known to possess antimalarial activity, Neuwinger (1996) reports the use of *A. secundiflora* and of *A. lateritia* in Tanzania and Kenya for treating malaria related symptoms. Brossat *et al*. (1981) found *A. vaombe* to have minimal *in vitro* antimalarial activity (82.2 ± 0.7% growth over 78h); whereas *in vivo* the aqueous extract was found to be a non-specific immunostimulant clearing a *P. berghei* malaria infection within two days if used prophylactically before the mice were inoculated. This,

together with the reality of resistant strains of malaria which continue to emerge and plague the African continent, encouraged us to further investigate the antiplasmodial properties of *Aloe* species, a genus which has gained a reputation as a cure-all group of plants including traditional use in treating malaria.

Materials and Methods

Plant material (leaves or roots) of 34 species of *Aloe*, representative of the chemotypes defined by Viljoen (1999) were collected at the National Botanical Institute (Pretoria) and dried at 28°C for 5 days. The dried leaves were ground and 1g of the powdered material extracted with methanol for 24h. As the primary interest was to assess the activity of the polar anthrone and chromone glycosides contained in the leaf exudate only methanol extracts were prepared. After filtration the methanol was evaporated and all samples reconstituted in DMSO to the desired initial concentration.

Samples were dissolved in methanol and passed through Phenomenex C18 cartridges. These purified samples were dissolved in methanol-water (1:1) and injected into the HPLC system. Operating conditions were as follows: A Phenomenex IB-Sil column was used (C18 reverse phase, 5µm particle size, 250mm x 4.6mm internal diameter; flow rate 1ml min^{-1}; 20 Fl sample loop). The solvent system comprised a 30% to 60% linear gradient of methanol in water over 25min, 3min isocratic, 100% in 2min, 4min isocratic. Detection was by diode array detector, using two channels (A set at 275 ± 70nm; B set at 365 ± 40nm). Compounds were tentatively identified by comparison of the retention

times and UV/VIS spectra with reference samples. Authentic reference samples were made available through previous studies (Viljoen 1999).

The chloroquine-resistant Gambian FCR-3 strain was cultured *in vitro* according to the method described by Jensen and Trager (1976). Briefly, parasitised erythrocytes were suspended at a 5% haematocrit in RPMI-1640, supplemented with 10mM D-glucose, 0.32mM hypoxanthine, 50mg l⁻¹ gentamicin, 10% (v/v) heat inactivated human plasma and was buffered with 25mM HEPES and 25mM $NaHCO_3$. Cultures were maintained at 37°C with a gas mixture of 5% CO_2, 3% O_2 and the balance with N_2. Cultures were synchronised with 5% D-sorbitol when the parasites were in the ring stage (Lambros and Vanderberg 1979). The percentage parasitaemia and stages were assessed daily by microscopic examination of thin blood smears stained with Giemsa.

The antimalarial activity of the various *Aloe* extracts was determined using the tritiated hypoxanthine incorporation assay (Desjardins *et al*. 1972). The parasite suspension, consisting of predominately the ring stage, was adjusted to a 0.5% parasitaemia and 1% haematocrit and exposed to the various concentrations of *Aloe* extracts for a single (48h) and double (78h) cycle of parasite growth. All assays were carried out using untreated parasites and uninfected red blood cells as controls. Labelled ³H-hypoxanthine (0.5µCi/well) was added after 24h and the cells were harvested at the appropriate time on a GFB-filtermat with a Titertek® cell harvester. The filtermats were dried, transferred to sample bags which were filled with scintillation cocktail and sealed. The concentration that inhibits 50% of parasite growth (IC_{50} value) was determined from the sigmoid dose response curve generated by the Enzfitter® and Prism® software. Chloroquine and quinine were used as the reference antimalarial agents.

For the toxicity assays Graham cells (transformed human kidney epithelium cells) and GCT (giant cell tumour) lung cancer cells were maintained continuously in culture at 37°C in 5% CO_2. The MTT (3-[4,5-dimethylthiazol-2yl]-2,5diphenyltetrazolium bromide) cellular viability assay was used to determine the toxicity profile of the extracts (Mossmann 1993). The trypsinised cell suspension was adjusted to 0.5 million cells ml⁻¹ and plated out with the *Aloe* extracts. The extracts were tested at the same concentrations used in the antimalarial assay. After 44h of incubation, 2mM MTT was added to the plates and incubated for a further 4h. DMSO was added to stop the reaction and to dissolve the formazan crystals. The absorbance was read at the test wavelength of 540nm and reference wavelength of 690nm and the percentage cellular viability calculated with appropriate controls taken into account.

Results and Discussion

The 34 *Aloe* species tested showed variable antimalarial activity. *Aloe excelsa*, *A. bulbilifera*, *A. ramosissma*, *A. arborescens*, *A. kedongensis* and *A. esculenta* showed minimal inhibitory activity (10–20%) over a double cycle (78h) of parasite growth. *Aloe andongensis*, *A. microstigma*, *A. mitriformis*, *A. verdoorniae*, *A. spicata*, *A. suzannae, A morijensis* and *A. tidmarshii* were slightly more active by inhibiting

approximately 40% of parasite growth over the double cycle. These species are chemically diverse and no obvious correlation could be found between the recorded activity (or lack thereof). The chemical profiles are summarised in Table 1. *Aloe microstigma* produces a suite of anthrone derivatives (Viljoen and Van Wyk 2001) while *Aloe mitriformis* accumulates two cinnamoyl chromones as the major metabolites (Viljoen and Van Wyk 1999). These two species also differ markedly in chemical composition when compared to the flavonoid-producing *Aloe suzanne* (Viljoen *et al*. 1998), the plicatolside-producing *A. morijensis* (Viljoen *et al*. 1999) and the aloin-accumulating *A. spicata* (Viljoen and Van Wyk 2001).

The leaf extracts of *Aloe viridiflora, A. wickensii, A. speciosa* and *A. suprafoliata* exhibited the most promising antimalarial activity over a single and double cycle of growth (Table 1), with *A. viridiflora* being the most active (Figure 1). HPLC analysis of these species (Figure 2) indicates the presence of the anthrone *C*-glucoside homonataloin A and B to be present in all four species. It is interesting to note that *A. mitriformis* also contains homonataloin but in much lower quantities (20%) compared to the *ca.* 50–60% homonataloin recorded for the four most active species.

As most species of *Aloe* produce either aloin or homonataloin as the major anthrone *C*-glycoside (Viljoen 1999), the activity of these two compounds were tested. Pure homonataloin was found to be approximately 8 times more active than the aloin standard over a single and double cycle of parasite growth in both chloroquine-resistant and -sensitive malaria parasites (Table 2). The chloroquine-sensitive 3D7 strain was more resistant to the extracts than the chloroquine-resistant FCR-3 strain with IC_{50} values for aloin of 169.76 ± 11.5µg ml⁻¹ and 109.05 ± 2.21µg ml⁻¹ over a single and double cycle of growth, respectively. IC_{50} values obtained for homonataloin were 18.24 ± 1.16µg ml⁻¹ and 15.43 ± 0.47µg ml⁻¹ over a single and double cycle of growth, respectively.

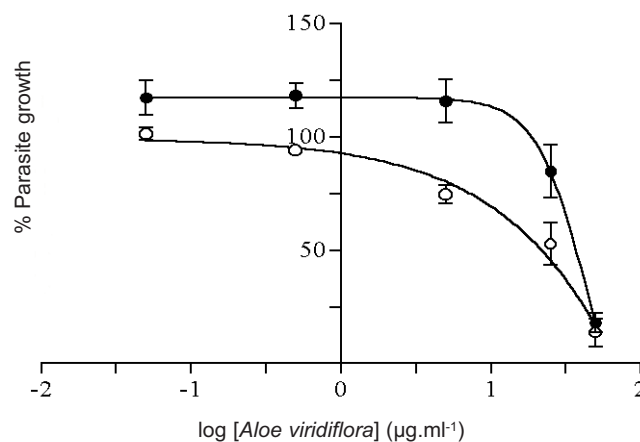The genus *Aloe* is characterised by a strong distinction

Figure 1: The sigmoid dose response curve showing the antimalarial activity of *Aloe viridiflora* on the *in vitro* growth of *Plasmodium falciparum* over a single (●) and double (○) cycle of parasite growth

Table 1: Anti-plasmodial activity and leaf exudate chemistry of *Aloe* species. 1 = aloesin, 2 = 7-hydroxyaloesin, 3 = aloenin, 4 = homonataloside B, 5 = 10-hydroxyaloin B, 6 = 8-*O*-methyl-7-hydroxyaloin, 7 = 3'-*O*-coumaroylaloesin, 8 = aloeresin A, 9 = plicataloside, 10 = microstigmin, 11 = aloeresin E, 12 = isovitexin, 13 = 5-hydroxyaloin B, 14 = broomii chromone I, 15 = 10-hydroxyaloin-6'-monoacetate, 16 = aloeresin D, 17 = aloeresin F, 18 = aloin A & B, 19 = 6-*O*-coumaroylaloesin, 20 = homonataloin A & B, 21 = deacetyllittoraloin, 22 = aloinoside A & B, 23 = littoraloin, 24 = microdontin A & B, 25 = flavanones, 26 = nataloin A & B, 27 = broomii chromone II, 28 = 3'6'-di-*O*-coumaroylaloesin

Species	% Parasite growth at 50µg ml^{-1}						Exudate chemistry
	Single cycle			Double cycle			
	%	s.d.[1]	n	%	s.d.	n	
A. andongensis[2]	87.13	3.95	5	67.17	17.09	6	
A. arborescens	90.27	11.86	2	81.27	0.07	2	1,3, 16, 18
A. barberae[2]	90.78	11.99	2	78.40	6.87	2	
A. boylei	87.74	23.21	4	70.60	23.78	4	12
A. broomii	97.29	1.58	2	72.93	6.53	2	1, 14, 13, 18, 10, 7
A. bulbilifera[2]	96.30	10.73	3	90.78	2.67	2	
A. capitata var. *gneissicola*	96.09	0.89	2	79.24	0.88	2	1, 2, 18
A. castanea	93.52	0.08	2	70.20	2.67	2	1, 18, 19
A. commixta	102.04	23.79	2	91.33	10.19	2	1, 12, 18
A. deyeri[2]	92.33	12.18	2	77.52	2.92	2	
A. dichotoma[2]	92.34	3.85	3	72.35	18.08	3	
A. divaricata[2]	93.72	12.45	3	80.77	15.58	2	2, 18
A. esculenta	91.98	7.66	3	80.65	13.78	3	2, 5, 15, 18, 21, 23
A. excelsa	96.27	8.32	3	90.23	13.46	2	1, 2, 8, 16, 18
A. globuligemma[2]	83.26	11.90	4	73.75	10.51	3	1, 2, 16
A. juvenna[2]	89.46	6.99	2	76.08	9.45	2	
A. karasbergensis[2]	101.49	10.37	2	75.10	12.08	2	
A. kedongensis[2]	91.40	6.23	2	79.69	1.03	2	1, 2, 3, 26
A. marlothii (leaves)	92.28	10.63	2	81.81	6.37	2	1, 8, 16, 18
A. marlothii (roots)	81.37	15.80	4	64.26	9.19	3	Van Wyk *et al.* (1995)
A. microstigma[2]	86.54	8.78	6	66.17	8.66	6	1, 2, 10, 13
A. mitriformis	84.57	8.61	3	61.07	14.62	4	1, 8, 11, 17, 20
A. morijensis	82.86	2.69	2	69.51	6.17	2	9
A. ramosissima[2]	88.92	19.71	2	85.36	2.06	2	
A. secundiflora[2]	90.28	1.64	2	65.97	1.85	2	3, 16, 18
A. spicata[2]	92.18	7.42	3	63.95	11.50	3	1, 18, 19
A. speciosa[2]	64.24	7.59	4	44.81	14.69	5	20
A. succotrina[2]	100.66	5.73	2	78.83	3.42	2	1, 22
A. suprafoliata	65.19	14.95	4	50.46	17.79	4	1, 8, 20
A. suzannae	96.00	1.34	2	68.50	17.18	3	12, 25
A. tidmarshii[2]	90.22	5.18	2	68.82	4.19	2	12
A. vaombe[2]	90.81	18.74	2	82.18	0.70	2	6
A. verdoorniae[2]	85.24	14.67	3	63.73	6.46	3	
A. viridiflora	16.71	4.18	4	9.83	4.46	5	1, 8, 20
A. wickensii	49.67	12.00	5	45.06	16.64	5	1, 4, 7, 20

[1] s.d. = standard deviation
[2] Species contain in addition to compounds 1–28 various compounds which have not been identified and only have a restricted occurrence in *Aloe*

Table 2: The antimalarial activity and toxic effects of the *Aloe* extracts in comparison to the reference antimalarial agents, chloroquine and quinine

| *Aloe* extract	Antimalarial activity IC[50] value (µg ml^{-1})		% Cellular viability at 50µg ml^{-1}	
	Single cycle	Double cycle	Graham cell line	GCT cell line
A. viridiflora	31.71 ± 5.10	21.83 ± 5.71	100.07 ± 15.18	121.20 ± 4.37
A. wickensii	55.06 ± 10.34	44.9 ± 9.64	90.24 ± 3.75	99.65 ± 6.28
A. speciosa	76.76 ± 15.89	45.00 ± 18.06	94.65 ± 19.35	109.41 ± 2.16
A. suprafoliata	70.45 ± 18.93	54.74 ± 19.05	88.43 ± 12.95	119.15 ± 5.06
A. marlothii (roots)	>50	55.74 ± 22.66	91.14 ± 8.87	106.12 ± 1.58
Aloin	107.20 ± 4.14	57.14 ± 5.65	98.39 ± 3.57	n.d.
Homonataloin	13.46 ± 1.36	9.22 ± 3.07	100.00 ± 3.57	n.d.
Chloroquine	0.09 ± 0.02	0.01 ± 0.01	98.00 ± 2.86	n.d.
Quinine	0.13 ± 0.04	0.03 ± 0.01	91.07 ± 4.67	n.d.

n.d. = not determined
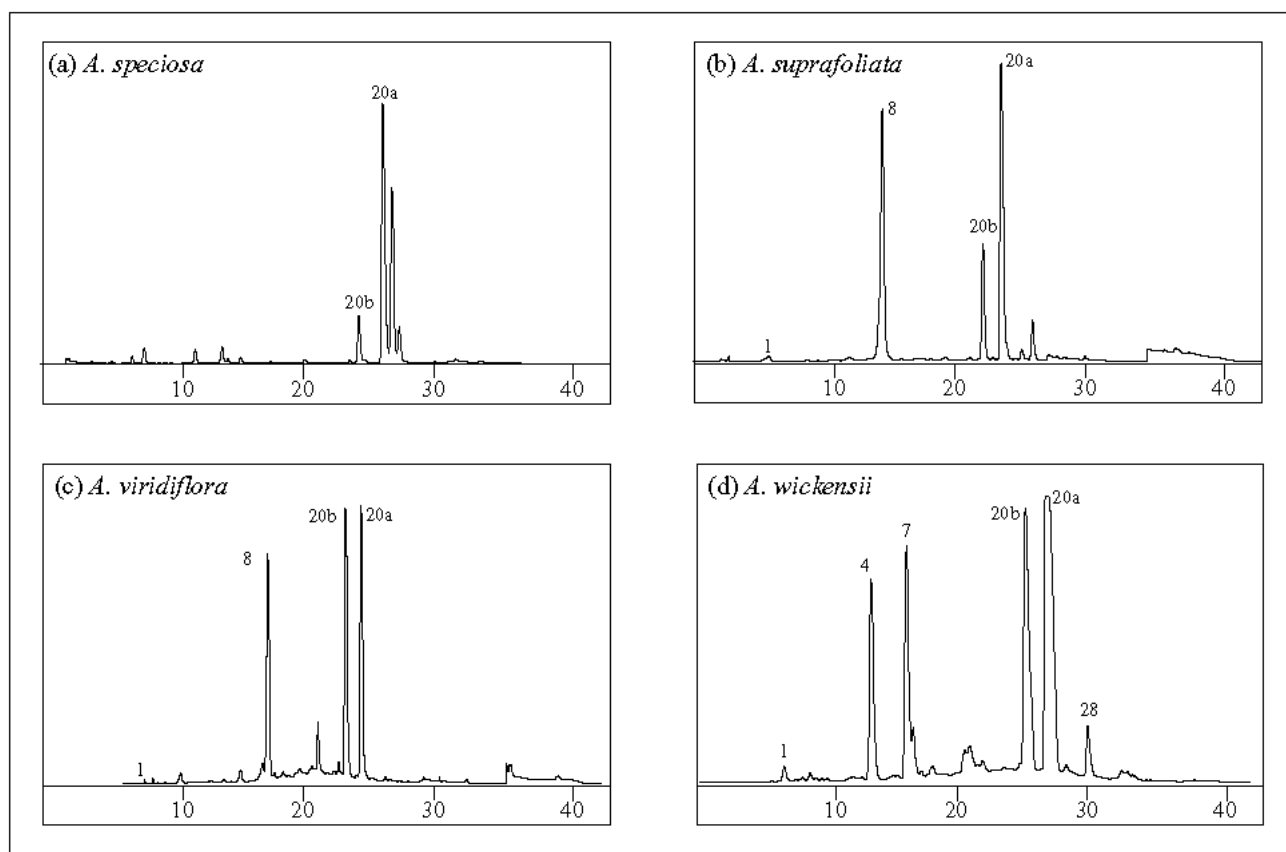
Figure 2: The HPLC chromatograms for the most active species showing the presence of the anthrone C-glucoside homonataloin common to *Aloe viridiflora, A. wickensii, A. speciosa* and *A. suprafoliata*. Peak numbers correspond to compounds in Table 1

between subterranean and above ground metabolism (Dagne *et al*. 2000). For this reason we also included the root extract of *A. marlothii* which possessed some inhibitory activity which was more pronounced over a double cycle of growth (Table 1). In comparison to the 42.3% inhibitory activity of 50µg ml^{-1} of the root extract, the leaf extract only inhibited 18.2% parasite growth over a double cycle of growth. The roots of *Aloe* species generally accumulate anthraquinones, preanthraquinones and bianthraquinoids (Wyk *et al*. 1995, Dagne *et al*. 2000). It is interesting to note that we recently found emodin (an anthraquinoine) to inhibit the *in vitro* intra-erythrocytic growth of chloroquine-resistant FCR-3 malaria (S Belotti, pers. comm.).

Aloes are store houses of numerous medicinal compounds and it is noteworthy to report the antimalarial properties in a screening of 7% (34 out of 460 species) of this genus characterised by immense chemical complexity. Furthermore, it is encouraging that the species which exhibited inhibitory effects on the intra-erythrocytic growth of the malaria parasite are not toxic to human cell lines as presented in Table 2. Considering that natural molecules such as quinine and artemisinin have acted as natural templates in the development of antimalarial agents, we wish to encourage further investigations into *Aloe* constituents in search for potent antiplasmodial agents.

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References

Agarwal SK, Singh SS, Verma S, Kumar S (2000) Antifungal activity of anthraquinone derivatives from *Rheum emodi*. **Journal of Ethnopharmacology 72**: 43–46

Brossat JY, Leduaut JY, Ralamboranto L, Rakotovao LH, Solar S, Gueguen A, Coulanges P (1981) Immunostimulating properties of an extract isolated from *Aloe vahombe*. 2. Protection in mice by fraction F1 against infections by *Listeria monocytogenes*, *Yersinia pestis*, *Candida albicans* and *Plasmodium berghei*. **Archives de L'Institut Pasteur de Madagascar 48**: 11–34

Camacho MR, Kirby GC, Warhurst DC, Croft SL, Phillipson JD (2000) Oxoaporphine alkaloids and quinones from *Stephania dinklagei* and evaluation of their antiprotozoal activities. **Planta Medica 66**: 478–480

Dagne E, Bisrat D, Viljoen AM, Van Wyk B-E (2000) The chemistry of *Aloe* species — A review. **Current Organic Chemistry 4**: 1055–1078

Desjardins RE, Carfield CJ, Haynes DJ, Chulay JD (1972) Quantitative assessment of antimalarial activity *in vitro* by semi-automated microdilution technique. **Antimicrobial Agents and Chemotherapy 16**: 710–718

Jensen JB, Trager W (1976) Human malaria parasites in continuous culture. *Science* **193**: 674–675

Lambros C, Vanderberg P (1979) Synchronisation of *Plasmodium falciparum* erythrocytic stages in culture. ***Journal of Parasitology*** **65**: 418–420

Mossmann T (1993) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. ***Journal of Immunological Methods 1993***, **65**: 55–63

Neuwinger HD (1996) African Ethnobotany. Poisons and Drugs. Chapman & Hall, London, UK, pp 3–9

Rojas L, Matamoros M, Garrido N, Finlay C (1995) The action of an aqueous extract of *Aloe barbadensis* Miller in an *in-vitro* culture of *Trichomonas vaginalis*. ***Revista Cubana de Medicinia Tropical*** **47**: 181–184

Semple SJ, Pyke SM, Reynolds GD, Flower RL (2001) *In vitro* antiviral activity of the anthraquinone chrysophanic acid against poliovirus. ***Antiviral Research*** **49**: 169–178

Sydiskis RJ, Owen DG, Lohr JL, Rosler KH, Blomster RN (1991) Inactivation of enveloped viruses by anthraquinones extracted from plants. ***Antimicrobial Agents and Chemotherapy*** **35**: 2463–2466

Van Wyk B-E, Yenesew A, Dagne E (1995) Chemotaxonomic survey of anthraquinones and pre-anthraquinones in roots of *Aloe* species. ***Biochemical Systematics and Ecology*** **23**: 267–275

Viljoen AM (1999) A chemotaxonomic study of phenolic leaf compounds in the genus *Aloe*. PhD thesis, Rand Afrikaans University, Johannesburg, South Africa

Viljoen AM, Van Wyk B-E (1999) The chemotaxonomic value of two cinnamoyl chromones, aloeresin E and F in *Aloe* (Aloaceae). ***Taxon*** **48**: 747–754

Viljoen AM, Van Wyk B-E (2001) A chemotaxonomic and morphological appraisal of *Aloe* series *Purpurascentes*, *Aloe* section *Anguialoe* and their hybrid, *Aloe broomii*. ***Biochemical Systematics and Ecology*** **29**: 621–632

Viljoen AM, Van Wyk B-E, Newton LE (1999) Plicataloside in *Aloe* — a chemotaxonomic appraisal. ***Biochemical Systematics and Ecology*** **27**: 507–517

Viljoen AM, Van Wyk B-E, Van Heerden FR (1998) The distribution and chemotaxonomic significance of flavonoids in the genus *Aloe*. ***Plant Systematics and Evolution*** **211**: 31–42