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Plicataloside in *Aloe* – a chemotaxonomic appraisal

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Abstract

In a chemotaxonomic survey of 380 taxa of *Aloe*, 20 species were found to contain the naphthalene derivative plicataloside as the major phenolic in the leaf exudate. Most of these species are restricted to East Africa (Kenya, Uganda and Tanzania). Only three species (*A. chabaudii*, *A. palmiformis*, *A. plicatilis*) in southern Africa contained this compound while the Malagasy endemics studied were found to be devoid of plicataloside. Macromorphology of the species was examined to search for other characters common to the species, and taxonomic affinities are assessed. Previous studies have suggested some of the taxa defined by this unique chemical compound to be taxonomically related, while many of the taxa have not previously been associated together.

Keywords: Aloe; Aloaceae; Plicataloside; Chemotaxonomy

1. Introduction

Various studies have been undertaken to assess chromatographic patterns in the genus *Aloe* (Reynolds, 1985, 1986, 1990; and Cutler et al., 1980) but the chemotaxonomic utility of chemical characters at the level of infrageneric taxa has not been fully explored. A chemotaxonomic study of the genus *Aloe* has shown various chemical groups at the infrageneric level (Viljoen et al., 1995, 1998). The present classification system for the genus *Aloe*, which is largely artificial, emphasises the need for additional characters to be studied in a multidisciplinary approach. The leaf exudate chemistry is only additional to various other forms of taxonomic evidence that could be used to explore possible natural relationships.

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The work reported here is part of a general chemotaxonomic survey of the genus *Aloe*, and concentrates on findings related to distribution of plicataloside. This was first isolated from *A. plicatilis* (Wessels et al., 1996) and initially thought to be restricted to this anomalous species in the monotypic section *Kumara*.

2. Materials and methods

Leaf exudate was collected in situ and at the National Botanical Institute, Pretoria (NBI), and National Botanical Gardens, Kirstenbosch (NBG). Samples from east Africa were from the aloe collection of L.E. Newton and other succulent plant specialists. Species included in the discussion and voucher details are listed in Table 1.

The exudate and leaf extracts were investigated with HPLC. Samples were dissolved in methanol and passed through C_{18} cartridges to remove substances of high retention time. 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 (C_{18} reverse phase, 5 μm particle 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

Table 1
Plicataloside-containing species of *Aloe* and corresponding voucher details. Abbreviations: RBG and K = Royal Botanical Garden (Kew); NBI = National Botanical Institute (Pretoria); NBG = National Botanical Garden (Kirstenbosch); EA = National Museum of Kenya; M = Missouri Botanical Garden

Species	Voucher
A. archeri Lavranos	Newton 3118 (neotype plant) (K, EA)
A. babatiensis Christian and Verdoorn	RBG 1974-4463
A. chabaudii Schönland	Ellert 32 (ex Zimbabwe)
A. deserti Berger	Newton 3608 (type locality)
A. fibrosa Lavranos and Newton	ex hort P. Favell
A. francombei Newton	Newton 4130 (type plant) (K, EA)
A. labworana (Reynolds) S. Carter	RBG 295-58-2921
A. morijensis S. Carter and Brandham	Newton 3661 (K)
A. multicolor Newton	Newton 4133 (type plant) (K, EA)
A. murina Newton	Newton 2497 (type plant) (K, EA)
A. otallensis Baker	RBG 194-09-012941
A. palmiformis Baker	RBG 224-94-020-95
A. parvidens Gilbert and Sebsebe	RBG 1985-4219 & Newton 4384
A. plicatilis (L.) Miller	NBG 19503
A. pustuligemma Newton	Newton 3739 (type plant) (K, EA)
A. rugosifolia Gilbert and Sebsebe	Sebsebe 22
A. schweinfurthii Baker	ex hort NBI
A. tugenensis Newton and Lavranos	Newton 3514 (type plant) (K, EA, MO)
A. ukambensis Reynolds	NBI 20505
A. wredfordii Reynolds	RBG 1977-4192

isocratic, 100% in 2 min, 4 min isocratic. Detection was by diode array detector, using two channels (A set at 275 ± 70 nm; B set at 365 ± 40 nm). Compounds were identified by comparison of the retention times and UV/VIS spectra with reference samples.

3. Results and discussion

3.1. Leaf exudate chemistry

Of 380 taxa of *Aloe* in the chemotaxonomic survey, 20 species (Table 1) were found to have plicataloside in the leaf exudate. In most of the 20 species it was found that this is the only phenolic compound in the exudate (Fig. 2) detected under the analysing parameters. In some species this compound co-occurred with trace amounts of the other compounds characteristic of *Aloe* (the chromones and anthrones) or together with unidentified compounds. Previous phytochemical work on *Aloe* reported the presence of a dark purple staining zone (P35), which most probably correlates to plicataloside (methods given in Reynolds 1985). This zone was reported in *A. plicatilis*, *A. otallensis*, *A babatiensis* and *A. deserti* (Reynolds, 1985) and also in *A. palmiformis* and *A. fibrosa* (Reynolds, 1990). Our survey confirmed that plicataloside is present in these species.

Fig. 1. The geographical distribution of the plicataloside-containing species of *Aloe*.

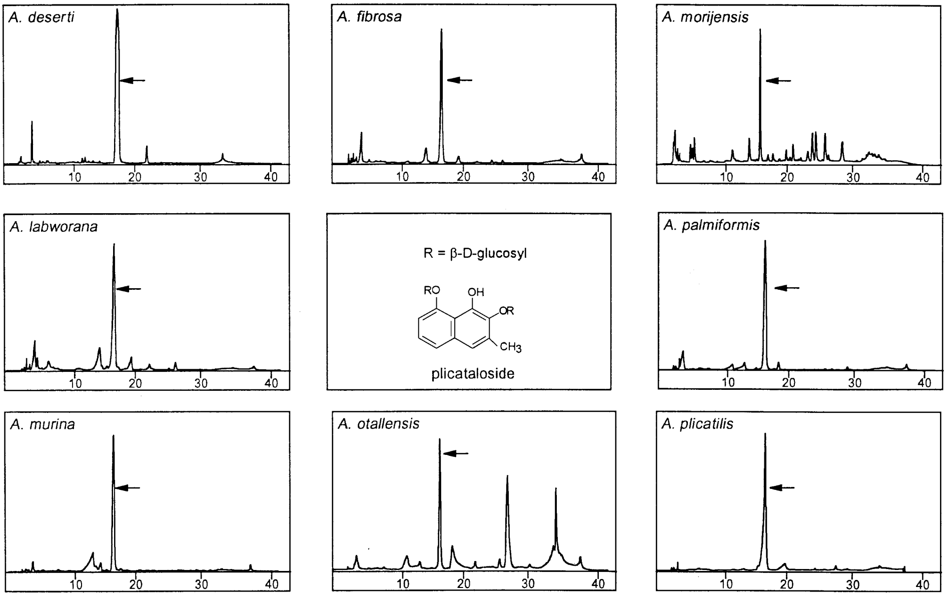

Fig. 2. A selection of plicataloside-containing species showing the unique HPLC profile. Plicataloside with a retention time of 17.8 min is denoted by the arrow. The structure of plicataloside has also been included.

3.2. Other characters

After the group of plicataloside-containing species had been identified, descriptions of these species were examined to see if there are other characters common to members of the group. The morphological characters are summarised in Table 2.

3.2.1. Habit

Several growth forms found in the genus *Aloe* are represented in the group. The basic habit forms are:

1.1. Acaulescent or with a short procumbent stem: *A. chabaudii*, *A. labworana*, *A. schweinfurthii*, *A. rugosifolia*, *A. ukambensis*, *A. murina*, *A. otallensis*, *A. parvidens*, and *A. wrefordii*. The first five species listed above form dense groups due to suckering from the base. The other species are all solitary or occur in small groups.

1.2. Most species in this chemical group produce long stems that are initially erect but become decumbent as the length of the stem increases. *Aloe archeri* is the extreme situation where the decumbent stem could reach lengths of 4 m long. Longer stems of *A. deserti*, *A. fibrosa* and *A. morijensis* are frequently supported by surrounding shrubs. The other species in this category are: *A. babatiensis*, *A. multicolor*, *A. palmiformis*, *A. pustuligemma* and *A. tugenensis*.

1.3. A completely different growth form is presented by the South African species *Aloe plicatilis*, which develops a robust, dichotomously branched stem that remains erect, giving this aloe a tree-like appearance.

3.2.2. Leaf characters

Most species (14 out of 20) represented in this group bear their leaves in a spreading manner with the leaf deflexed or in some case only the apices are recurved. This applies to *A. archeri*, *A. babatiensis*, *A. deserti*, *A. fibrosa*, *A. labworana*, *A. morijensis*, *A. multicolor*, *A. otallensis*, *A. palmiformis*, *A. parvidens*, *A. pustulegema*, *A. schweinfurthii* and *A. tugenensis*. In *A. chabaudii*, *A. francombei*, *A. murina* and *A. plicatilis*, the leaves are erect and not decurved. *Aloe plicatilis* once again represents a distinctive difference as the leaves are distichously arranged while all other species have the leaves arranged in rosettes. In *A. rugosifolia* and *A. ukambensis* the leaves are slightly incurved. The latter species has leaves that are conspicuously striate; the only other species in this group with lineate markings is *A. chabaudii*. Most species in this group have leaves spotted on the upper or lower leaf surface or on both. With the exception of a few species the leaf margin is armed with large deltoid pungent teeth. The leaf surface is usually smooth, with the exception of *A. archeri*, *A. deserti*, *A. francombei*, *A. murina*, *A. rugosifolia* and *A. tugenensis*, all of which have a somewhat asperous leaf surface.

3.2.3. Bracts

Based on quantitative measurements two "size groups" are recognised within this group. Those species in which the bracts are very small (3–7 mm) are *A. chabaudii*, *A. labworana*, *A. murina*, *A. palmiformis*, *A. parvidens*, *A. plicatilis* and *A. schweinfurthii*. In all the other species the bracts are fairly large, ranging in length from 10 to 30 mm. The bracts of *A. deserti* and *A. rugosifolia* are distinctly white and deflexed.

3.2.4. Inflorescence and raceme

With the exception of the following species, most species in the group have a panicle with cylindrical racemes. *Aloe deserti* presents the unique phenomenon where the racemes are initially drooping and limp and become stiff and erect with maturity. In *A. fibrosa*, *A. morijensis*, *A. palmiformis*, *A. plicatilis* and *A. ukambensis* the inflorescence is not a much-branched panicle but mostly simple or 2–3–branched in some cases. *Aloe ukambensis* is the only member of this group that has a sub-capitate raceme. *A. murina* is also unique in the group as the developing buds are secund.

3.2.5. Perianth

Most species pertaining to this group have a perianth that is cylindrical-trigonous in shape. The following species deviate from this general pattern. *Aloe chabaudii* has a decurved perianth, which is restricted above the ovary, where it is trigonously indented. A sub-clavate to clavate perianth is characteristic of *A. otallensis*, *A. wrefordii* and *A. multicolor*. *Aloe pustuligemma*, *A. francombei* and *A. otallensis* are characterised by the pustulate perianth surface.

3.2.6. Distribution

As shown in Fig. 1, most species in this group are distributed in Tropical East Africa (Uganda, Tanzania and Kenya). The arm extending into West Africa represents only *A. schweinfurthii*. The highest number of plicataloside species (13) occur in

Table 2
Summary of salient morphological characters for the plicataloside-containing species of *Aloe*

Species	Habit	Suckering	Leaf orientation	Leaf surface	Bracts (mm)	Inflorescence	Perianth	Exudate chemistry
A. archeri	Long decumbent stem	No	Spreading and recurved	Unspotted rough	12–15	6–12-branched panicle	Cylindrical trigonous	Only plicataloside
A. babatiensis	Erect or decumbent	No	Spreading and recurved	Unspotted smooth	20–30	2–4-branched panicle	Cylindrical trigonous	Plicataloside and unidentified cmpds
A. chabaudii	Mostly acaulescent	Yes	Leaves erect	Obscurely lineate sometimes with a few spots	3–5	6–12-branched panicle	Decurved, trigonously indented	Plicataloside and unidentified cmpds
A. deserti	Erect/sprawling stem	No	Spreading becom-ing decurved	With or without spots rough	15 mm, deflexed	3–8-branched panicle	Cylindrical trigonous	Only plicataloside
A. fibrosa	Stem erect or decumbent	No	Erect apices often recurved	Unspotted smooth	12–18	Simple or 1–2-branched	Cylindrical, slightly restriced obove ovary	Only plicataloside
A. francombei	Shortly caulescent	No	Erect	Spotted rough	10–12	Up to 8-Branched panicle	Cylindrical trigonous	Plicataloside and traces of aloin
A. labworana	Acaulescent	Yes	Spreading, apices recurved	Densely spotted smooth	1.5–3	10–12-branched panicle	Cylindrical trigonous	plicataloside and traces of aloin
A. morijensis	Suberect, stem 1 m	No	Spreading to recurved	Spotted smooth	10–15	Simple or 1–2-branched	Cylindrical trigonous	Only plicataloside
A. multicolor	Erect or decumbent	No	Spreading and recurved	Sparsely spotted beneath smooth	10–13	5–8-branched panicle	clavate	Only plicataloside

A. murina	Acaulescent	No	Spreading	Unspotted rough	5–6	Up to 13-branched panicle	Cylindrical trigonous	Plicataloside and traces of aloin
A. otallensis	Acaulescent	No	Erect, slightly recurved	Sometimes spotted smooth	12 mm	Up to 12-branched panicle	Cylindrical to clavate and papillose	Only plicataloside
A. palmiformis	Stem 1–1.5 m long	No	Spreading and recurved	Usually spotted beneath smooth	2–3	Simple or 2–4-branched	Cylindrical trigonous	Plicataloside and traces of aloin
A. parvidens	Acaulescent	No	Spreading and recurved	Spotted smooth	5–6	2–10-branched panicle	Cylindrical trigonous	Plicataloside and an unidentified cmpd
A. plicatilis	Distinctly caulescent	No	Distichous	Unspotted smooth	8 mm	Simple	Cylindrical trigonous	Only plicataloside
A. pustuli-gemma	Stems erect or decumbent	No	Spreading and recurved	Unspotted rough	11–13	8–9-branched panicle	Cylindrical and papillose	Only plicataloside
A. rugosifolia	Acaulescent	Yes	Erect and slightly incurved	Spotted rough	9–11, deflexed	8–10-branched panicle	Cylindrical trigonous	Only plicataloside
A. schweinfur-thii	Acaulescent	Yes	Spreading and recurved	Usually spotted near base smooth	4–7	8–10-branched panicle	Cylindrical trigonous	Only plicataloside
A. tugenensis	Decumbent stem-1 m	No	Spreading and recurved	Unspotted rough	10–12	Up to 12-branched panicle	Cylindrical trigonous	Plicataloside and unidentified cmpds
A. ukambensis	Acaulescent	Yes	Erect and slightly incurved	Striate smooth	7–10	Simple or 1–3-branched	Cylindrical trigonous	Plicataloside and unidentified cmpds
A. wrefordii	Acaulescent	No	Erect, apices recurved	Obscurely lineate smooth	9–12	Up to 16-branched panicle	Cylindrical clavate	Only plicataloside

Kenya. The two geographical "outliers" are *Aloe plicatilis* (South Africa) and *A. palmiformis* (Angola), which have localised distributions in their areas. Most species in this group have specific and localised distributions. *Aloe chabaudii*, *A. labworana*, *A. parvidens*, *A. schweinfurthii* and *A. ukambensis* deviate from the norm as they are widely distributed in comparison with the other species in this group.

3.3. Relationships

The "affinity diagram" (Fig. 3) is a summary of previously reported relationships between species in this group. All species contained in the solid block are characterised by possession of the naphthalene-like compound plicataloside, which is the unifying character for all the taxa.

In her Flora treatment of the Aloes of Tropical East Africa, Carter (1994) states that due to the absence of morphological characters indicating phylogeny, the species are not arranged in any phylogenetic sequence. She does however suggest that although the species are numerically arranged following the concept of Reynolds (1966), where species are basically arranged in terms of habit characters "from smallest to biggest", species that she thinks ‘belong together" are listed together (pers. comm.), though her list is not divided into groups. All the species in block A are closely arranged in the Flora treatment. The same applies for those species in block B. Yet, reference to

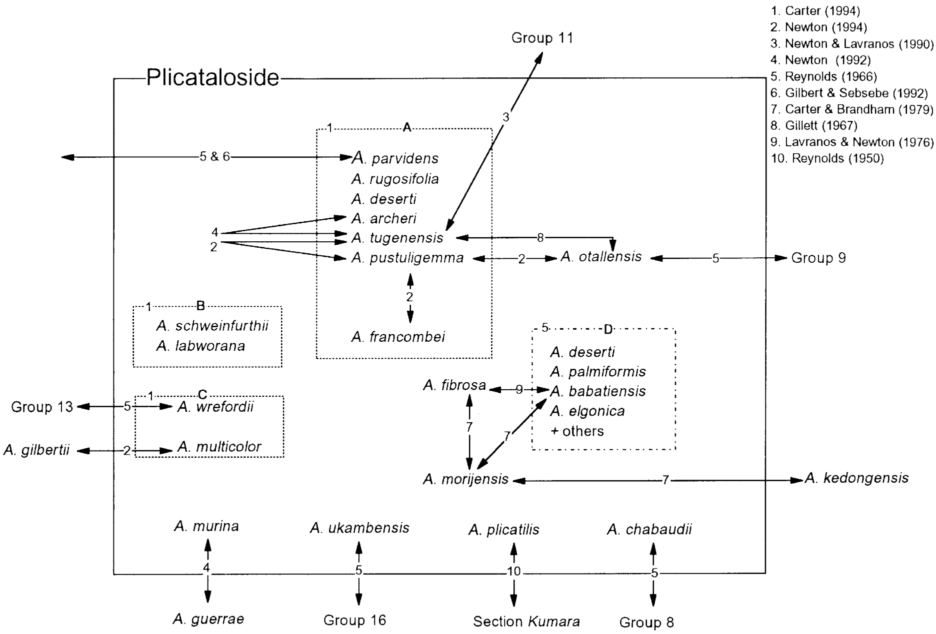

Fig. 3. A taxonomic "affinity-diagram" showing possible relationships between the taxa as previously suggested. Numbers correspond to literature references where these relationships between the taxa have been discussed.

literature shows that these two groups of species (block A and B) have not previously been associated with one another. Of the species listed in block A, many have been related to one another. Newton (1994) assessed the morphological relationships of some of these species. He remarks on the similar growth habit of *A. pustuligemma*, *A. archeri* and *A. tugenensis*, all having rough and unspotted leaves.

In the protologue of *A. tugenensis* (Newton and Lavranos, 1990), it is suggested that this species is closely related to *A. compacta* (now *A. macrosiphon* – unfortunately no exudate material of this species could be obtained). The authors speculate that the Group 11 created by Reynolds (1966) could include other species with large floral bracts that are imbricate and cover the flower buds, e.g. *A. deserti*. The latter species also contains plicataloside. In the amended species description of *A. archeri* (Newton, 1992) a correlation is drawn between *A. tugenensis* and *A. archeri* as both species have imbricate bracts, and they are similar in growth and habit. Three species in this plicataloside group (block A) are characterised by a pustulate perianth surface (*A. pustuligemma*, *A. otallensis and A. francombei*) and it has been suggested by Newton (1994) that it seems unlikely that this "unique character" would evolve more than once in the very small geographical area where all three species occur. Reynolds (1966) never saw *A. otallensis*, and as the other two allied species were described recently not too much emphasis should be placed on the present taxonomic position of this species, which places it in Group 9. *Aloe rugosifolia* was previously known as *A. otallensis* var. *elongata*. After assessing the identity of *A. otallensis*, Gilbert and Sebsebe (1992) raised this variety to specific rank and renamed it. As suggested by the new epithet, the leaves of this species are somewhat rugose. Rough leaves are also characteristic of *A. archeri*, *A. deserti*, *A. francombei*, *A. murina* and *A. tugenensis*. Another character placing *A. rugosifolia* close to *A. deserti* is the whitish, deflexed bract that is characteristic of both species.

Block B in Fig. 3 contains two species that are closely arranged in Carter (1994). *A. labworana* was previously described as a variety of the widespread *A. schweinfurthii*; hence the expected similarity.

A. wrefordii and *A. multicolor*, shown as block D in Fig. 3, also appear close together in Carter's account. *A. wrefordii* is placed by Reynolds (1966) in Group 13 as it has a clavate perianth. In this plicataloside group, two other species, *A. multicolor* and *A. otallensis*, have sub-clavate perianths. Newton (1994) suggests *A. multicolor* is most closely related to *A. gilbertii*. Although these two species differ in growth habit, leaf and bract characters this similarity is suggested on the basis of perianth shape.

The last significant group in the affinity diagram is contained in block D. Reynolds (1966) created Group 19 to house all species with a shrubby growth form. Four of the plicataloside species are included in this large group. Based on habit and inflorescence characters, *A. fibrosa* is suggested to be most closely related to *A. babatiensis* (Lavranos and Newton 1976). Both these species are in turn suggested to be closely allied to *A. morijensis*, while the latter species also shows a morphological resemblance to the non-plicataloside species, *A. kedongensis* (Carter and Brandham, 1979). The multi-disciplinary study by Cutler et al. (1980) on the morphology, anatomy, cytology and chemistry of a large group of species suggests an affinity between *A. fibrosa*, *A. morijensis* and *A. babatiesis*. The hypothesis is also put forward that *A. morijensis*, through chromosome doubling, could have given rise to the tetraploid species (*A. dawei* and *A. elgonica*). *A deserti* is placed in Group 19 by Reynolds (1966), but all the species with which it is associated in Block A, based on Carter (1994), were published after 1966.

All the species discussed above show some degree of morphological coherence with other plicataloside-containing species. Three of the four "peripheral" species have not previously been regarded as even distantly related to any other plicataloside-containing species. Newton (1992) suggests an affinity between *A. murina* and the Angolan endemic, *A. guerrrae*. Unfortunately no leaf exudate sample could be obtained from this species. The only other species in Angola to contain plicataloside is *A. palmiformis*.

Reynolds (1966) places *A. ukambensis* in group 16 of the tropical aloes. This is the only species in the "plicataloside-group" with a sub-capitate raceme. All the other species have cylindrical racemes. Together with *A. chabaudii*, these two species are also unique in that they are the only two species in which the leaves are striate. *Aloe chabaudii* is placed in group 19, which contains all species in which the perianth is trigonously indented. *Aloe parvidens* (syn. *A. pirottae* sensu Reynolds) is included in group 4 of the tropical aloes (Reynolds, 1966), which is defined as a group containing all species with "more-or-less" striped flowers. The last and most fascinating in terms of geographical isolation is the South-African endemic *A. plicatilis*, which is morphologically unique; hence its position in the monotypic section *Kumara* (Reynolds, 1950).

4. Conclusions

It would be presumptuous to suggest any evolutionary or phylogenetic hypotheses on the data presented. The presence of this interesting chemical compound in the 20 species, and the absence of the chromones and anthrones (characteristic of *Aloe*), encourages some speculation on the possibility that this could be a monophyletic group. However, this statement is not convincing as there is only one single chemical apomorphy for this group, and no morphological character could be found to re-enforce the chemical coherence shown by the leaf exudate chemistry. This comes as no surprise as it is known that taxonomic research on *Aloe* is always confronted by the reality that the genus has relatively few morphological apomorphies at the infrageneric level. This is best represented in the work of Reynolds (1966), where an utilitarian approach was followed to group "morphologically similar" species together, which obviously does not necessarily reflect or predict natural relationships. We do not wish to suggest that chemical characters should enjoy preference over the morphological characters as all problems (e.g. convergence) encountered with morphological characters are prevalent for chemical characters. However, we do believe that the presence of this unique compound should not be completely dismissed as chemotaxonomic coincidence, and we should at least explore further the possibility of taxonomic affinity between these plicataloside-containing taxa.

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