Short Communication / Kort Mededeling

Screening for cardiac glycosides in *Schizobasis intricata*

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Traditional herbalists in Natal use *Schizobasis intricata* (Bak.) Bak. as a substitute for *Bowiea volubilis* Harv. ex Hook. This may be due to morphological confusion of the two species or they may elicit a similar biological response. *Bowiea* bulbs are known to contain cardiac glycosides. A phytochemical screening of *S. intricata* for cardiac glycosides was carried out. *S. intricata* contains 2-deoxy sugars, common components in the sugar moieties of cardiac glycosides. Comparative chromatographic screening showed that *S. intricata* bulbs do not contain cardenolides nor any *Bowiea*-type bufadienolides. Other unknown bufadienolides may, however, be present in this plant.

Tradisionele kruiedoktors in Natal gebruik *Schizobasis intricata* (Bak.) Bak. as 'n alternatief vir *Bowiea volubilis* Harv. ex Hook. Dit mag die gevolg wees van morfologiese verwarring of die twee spesies mag dieselfde biologiese reaksie tot gevolg hê. Dit is bekend dat *Bowiea* kardiale glikosiede bevat. 'n Fitochemiese ondersoek is gedoen op *S. intricata* vir kardiale glikosiede. *S. intricata* bevat 2-deoksi-suikers, 'n algemene komponent van kardiale glikosiede. Vergelykende chromatografiese ondersoeke het getoon dat *S. intricata* bolle nie kardenoliede of enige *Bowiea*-tipe bufadiënoliede bevat nie. Ander onbekende bufadiënoliede mag egter in hierdie plant teenwoordig wees.

Keywords: *Bowiea volubilis*, cardiac glycosides, Hyacinthaceae, medicinal plant, *Schizobasis intricata*

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The report that *Schizobasis intricata* (Bak.) Bak. is used medicinally as a substitute for *Bowiea volubilis* Harv. ex Hook. (Drewes *et al.* 1993) aroused our curiosity as it was reported that *S. intricata* is edible, whereas *B. volubilis* is toxic (Dyer 1976). When a plant is reported to be toxic it is often an indicator that the plant contains pharmacologically active compounds; and vice versa, an edible plant is less likely to contain pharmacologically active compounds. In this report we investigated whether there is a chemical basis for the substitution of *B. volubilis* with *S. intricata* by traditional healers.

Both species are members of the Hyacinthaceae. Both are curious in that the leaves are reduced to bulb scales in the adult plant and the aerial part of the plant comprises a photosynthetic inflorescence. In *Bowiea* the inflorescence is twining whereas in *Schizobasis* it is erect (Figure 1).

As there are certain similarities in the appearance of the two plants, the possibility exists that the two species could be confused. *B. volubilis* is collected and marketed under the Zulu name iGibisila or iGuleni, and *S. intricata* under the Zulu name umThondo-wemfene (Cunningham 1988), but most herbalists in Natal seem not to know *S. intricata* or to confuse it with *B. volubilis* (Drewes *et al.* 1993).

Cunningham (1988) classified *B. volubilis* as 'declining' and *S. intricata* in the not-so-threatened group 'rare and vulnerable', so a reason for the substitution of *B. volubilis* with *S. intricata* could be the unavailability of *B. volubilis*, in which case *S. intricata* might be chosen as an alternative.

Bowiea volubilis has been used by different African ethnic groups as a purgative, against dropsy and as a cure for barrenness or externally for sore eyes and skin diseases, as well as for various magical purposes (Watt & Breyer-Brandwijk 1962). *S. intricata* bulbs were reported to be used in the same way as *B. volubilis* by herbalists (Drewes *et al.* 1993), but the plant is not mentioned by earlier ethnobotanists as being used medicinally.

Bowiea is known to contain cardiac glycosides. A number of bufadienolide-type glycosides specific for *Bowiea* have been isolated (Katz 1953, 1957; Tschesche *et al.* 1955; Tschesche & Dölberg 1958). We therefore carried out a comparative phytochemical screening for cardiac glycosides in *S. intricata* using *B. volubilis* extract for reference purposes. The sugar moieties of cardiac glycosides often contain unusual 2-deoxy sugars. The aglycone contains an unsaturated lactone ring: either a 5-membered ring, in which case the compound is known as a cardenolide, or a 6-membered ring, known as a bufadienolide. The cardenolides are used in western medicine as cardio-active drugs, whereas bufadienolides find little use as cardiac drugs because of their low therapeutic index and unacceptable side effects (Rang & Dale 1987). However, bufadienolides are used as expectorants and can induce vomiting (Rang & Dale 1987).

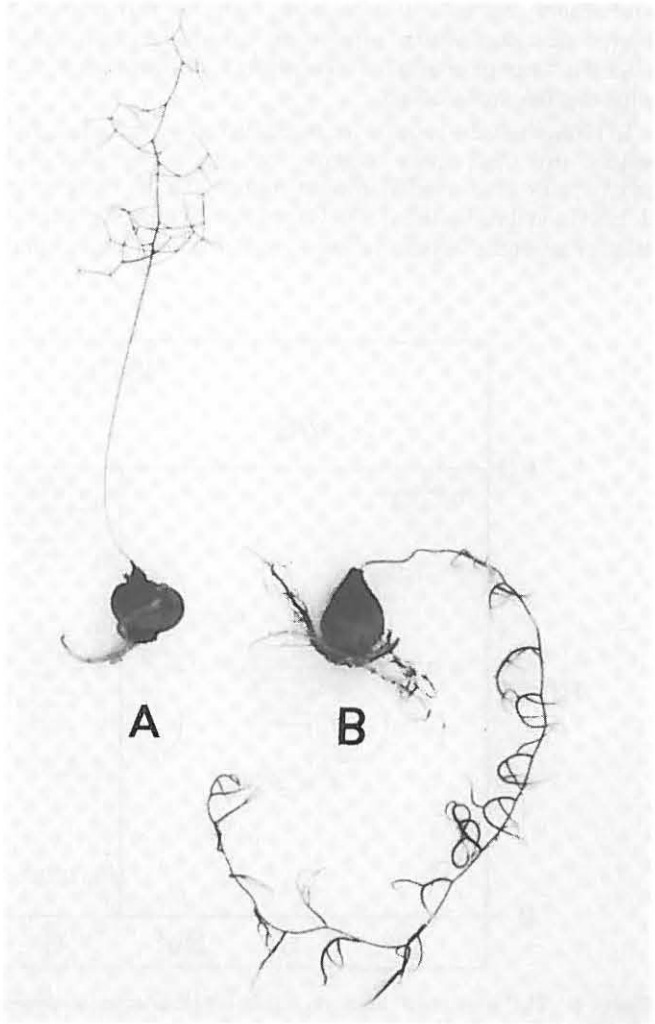

Figure 1 A. *Schizobasis intricata*. B. *Bowiea volubilis*. In *S. intricata* the inflorescence is erect, whereas in *B. volubilis* it is twining.

S. intricata bulbs were obtained from Silverglen Nature Reserve, Natal; these were 2–3 cm in diameter and the upper part of the bulb scales were greenish in colour. Bulbs of *B. volubilis* were produced by *in vitro* techniques in our laboratory; these were 2 years old, about 1cm in diameter and white in colour.

The bulbs were cut into pieces and dried at 60°C. The dried material was ground in a mortar and 1 g material was extracted by boiling for 15 min with 30 ml 53% ethanol containing 3% lead acetate. The extracts were cooled and filtered. The filtrate was acidified with acetic acid and partitioned three times in succession against 15 ml dichloromethane. The combined dichloromethane phases were dried with anhydrous sodium sulphate and the solvent was removed under vacuum. The residue was redissolved in 1 ml dichloromethane:ethanol 1:1.

To screen for 2-deoxy sugars using the Keller-Killiani test (Trease & Evans 1983), 500 µl redissolved residue was taken to dryness and redissolved in 1.5 µl glacial acetic acid containing 2.2 mM $FeCl_3$. This mixture was layered on top of 1 ml concentrated sulphuric acid. A brown ring appeared at the interface in both *B. volubilis* and *S. intricata*, and the upper phases turned blue, later dark brown, indicating the presence of 2-deoxy sugars. Digitoxin and digoxin were used as references.

In order to screen for the unsaturated lactone ring, 50-µl aliquots of each extract were applied to Merck Silica 60 F_{254} TLC plates. 10 µg digitoxin and digoxin were used as references. The TLC plates were developed in ethyl acetate:methanol:water 81:11:8. One plate was sprayed with Kedde's reagent (alkaline 3,5-dinitrobenzoic acid) (Wagner *et al.* 1984) which detects cardenolides, but not bufadienolides. Only the two references showed the characteristic purple-pink colours of cardenolides. From this it can be concluded that neither *S. intricata* nor *B. volubilis* contains cardenolides.

To further test the extracts for bufadienolide-type cardiac glycosides, two other spray reagents were used. One plate was sprayed with antimony(III)chloride reagent ($SbCl_3$) (Wagner *et al.* 1984) and then heated at 100°C for 6 min. With $SbCl_3$, bufadienolides generally appear yellow to yellow-brown under ultraviolet light of wavelentgh 366 nm. We found several bands in the *Bowiea* lane that appeared orange in UV light after spraying with $SbCl_3$ (Figure 2); this is in accordance with Tschesche and Dölberg (1957), who found that *Bowiea* glycosides appear yellow-orange under UV light with $SbCl_3$. *S. intricata* also contained several bands that appeared yellow or orange under UV light after spraying with $SbCl_3$; one or more of these bands could be a cardiac glycoside.

A further plate was sprayed with chloramine T–trichloroacetic acid reagent (Wagner *et al.* 1984) and heated for 10 min at 100°C. Chloramine T–trichloroacetic acid detects both cardenolides and bufadienolides and gives blue, yellow or yellow-green spots when viewed under UV light of 366 nm. In daylight all spots were brownish; viewed under 366 nm UV light, *Bowiea* bands appeared lemon yellow, whereas the bands in *Schizobasis* were yellow-orange (Figure 2).

It can be concluded that cardenolides are absent in *S. intricata* and *B. volubilis*. When combining information from all TLC plates (all results not shown), it could be seen that none of the bands in *B. volubilis* corresponded to those of *S. intricata*. *S. intricata* thus seems not to contain any of the *Bowiea* glycosides, and any rational use of *S. intricata* cannot be based upon a content of cardiac glycosides of the *Bowiea* type. However, *S. intricata* could contain other unknown bufadienolides.

Acknowledgements

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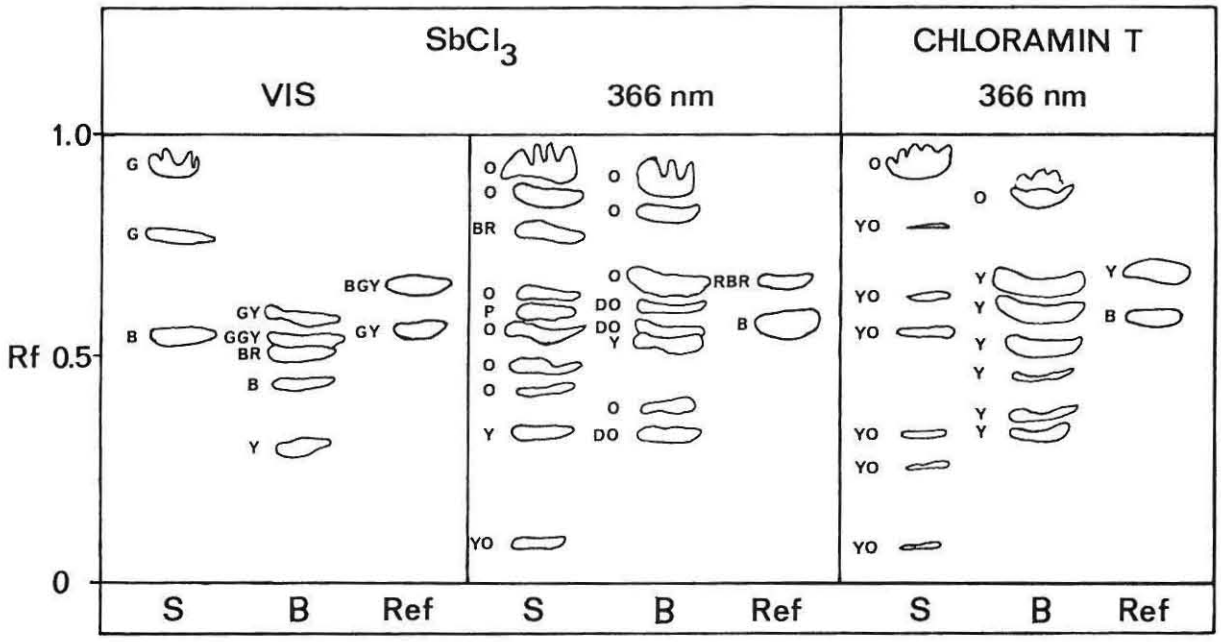

Figure 2 TLC plates of *S. intricata* and *B. volubilis* extracts sprayed with the cardiac glycoside-detecting reagents antimony(III)-chloride and chloramine T. S: *S. intricata* extract; B: *B. volubilis* extract; Ref: reference compounds, digitoxin at R_f 0.71, digoxin at R_f 0.62. Colours in the chromatogram: B: blue, BGY: blue-grey, BR: brown, DO: dark orange, G: green, GY: grey, GGY: green-grey, O: orange, P: purple, RBR: red-brown, Y: yellow, YO: yellow-orange.

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