Short Communications

Rhoicissus tridentata subsp. cuneifolia: the effect of geographical distribution and plant storage on rat uterine contractile activity

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Received 2 May 1999; revised 19 May 1999

Introduction
This study investigated the geographical variation in the contractile activity of crude water decoctions of Rhoicissus tridentata (L.f.) Wild & Drum. subsp. cuneifolia (Eckl & Zeyh). N.R. Urton, as used by traditional healers. The extracts were tested on isolated rat uterus. The results indicate that the uterotonic activity varies considerably depending on where the plant material was harvested. Most plant extracts stimulated varying degrees of contraction where the extract of one plant inhibited acetylcholine induced contractions. The effect of storing dried material at room temperature and away from sunlight was also investigated. Storing dried plant material does not alter the activity of crude water extracts.

Keywords: Rhoicissus tridentata, Vitaceae, spatial variation, distribution, storage, herbal remedy.

Rhoicissus tridentata (L.f.) Wild & Orum. subsp. cuneifolia (Eckl & Zeyh). N.R. Urton, is a deciduous shrubby creeper in the family Vitaceae, commonly known as wild grape (Smith 1966) or isiNwazi (Zulu) (Gersten 1941). Rhoicissus tridentata is a polymorphic species which has undergone numerous nomenclature revisions over the last two centuries. Five different species, R. cuneifolia, R. erythros, R. cirrhiflora, R. paniculiflora and R. dimidiata have been grouped together as R. tridentata, but the species has been divided into two subspecies, being subspecies tridentata and subspecies cuneifolia depending on the number of indentations on the leaflet margins (Urton et al. 1986).

Roots and tubers of Rhoicissus tridentata are widely used by Zulu traditional healers for stomach ailments, kidney and bladder complaints, epilepsy, infertility, dysmenorrhea, induction of labour and the facilitation of childbirth (Wat & Breyer-Brandwijk 1962; Bryant 1966; Pujol 1990; Hutchings et al. 1996). The use of the plant in pregnancy is the focus of this investigation which is the reason contractile activity on uterine tissue was measured.

Even though considerable work is being done to encourage traditional healers to grow their own medicinal plants (Mender et al. 1998), this is not yet the custom. Urbanisation has led to the generation of the industry of plant gathering. Formerly plant harvesting was done by the traditional healers themselves, however, urbanisation has increased the commercialisation of the herb trade. Now plant collecting is often done by untrained commercial gatherers who harvest plants from all over southern Africa and supply plants to Herbal Markets in urban areas (Cunningham 1988). Not only is this system having a disastrous effect on the populations of many popular plant species (Cunningham 1988), but seldom do healers know where or when the plant material was harvested (Pers comm).

The nature and quality of secondary metabolites can be affected by temperature, rainfall, aspect, length of day, light quality and altitude (Evans 1996). Therefore, this study was done to investigate whether there was any variation in the contractile activity of plants harvested from different localities, and whether storage altered the activity of the plant extracts. Both these possible areas of variation could impact on the effect manifested in the patients.

Lignotubers are the part used by traditional healers, but are very difficult to locate as they are interspersed along the roots which usually grow within crevices between rocks. Stem extracts were shown to have the uterotonic activity most similar to that of lignotuber extracts, following the same seasonal variation as lignotuber extracts (Katsoulis et al. 1999a). The contractile activity of root extracts was shown to be less than that of extracts from lignotubers and stems. Stems and roots were used for this investigation into the distributional variation in uterotonic activity of extracts from R. tridentata.

Methods
Plant collection - distributional variation
R. tridentata subsp. cuneifolia stems were collected during summer, which is the season during which the plants yield the most active aqueous extracts (Katsoulis et al. 1999a). The plant material was harvested from around the south eastern part of South Africa, from a variety of different habitats (Table 1). The identity of all specimens was verified by L. Katsoulis. Voucher specimens were lodged in the C.E. Moss Herbarium. (Specimen numbers given in Table 1).

Table 1 Collection details of plants harvested to determine the effect of distribution on the contractile activity of aqueous extracts

<table>
<thead>
<tr>
<th>Location (Voucher specimen number)</th>
<th>Province</th>
<th>Vegetation typea</th>
<th>Season</th>
<th>Part</th>
<th>Altitude (m)b</th>
<th>MARc (mm)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suikerbosrand Nature Reserve (086929)</td>
<td>Gauteng</td>
<td>Mixed grassveld</td>
<td>Autumn</td>
<td>stems</td>
<td>1541</td>
<td>698</td>
</tr>
<tr>
<td>Mondeer (3090060)</td>
<td>Gauteng</td>
<td>Sour grassveld</td>
<td>Autumn</td>
<td>stems</td>
<td>1541</td>
<td>698</td>
</tr>
<tr>
<td>Magaliesburg Mountains</td>
<td>Gauteng</td>
<td>Bushveld gorge</td>
<td>Summer</td>
<td>stems</td>
<td>1157</td>
<td>659</td>
</tr>
<tr>
<td>Umlazi (086931)</td>
<td>KwaZulu-Natal</td>
<td>Coast-belt scrub forest</td>
<td>Summer</td>
<td>roots</td>
<td>8</td>
<td>1022</td>
</tr>
<tr>
<td>Durban</td>
<td>KwaZulu-Natal</td>
<td>Coast-belt scrub forest</td>
<td>Summer</td>
<td>roots</td>
<td>8</td>
<td>1022</td>
</tr>
<tr>
<td>Morgenzaas Bos</td>
<td>Mpumalanga</td>
<td>Climax forest in sourveld gorge</td>
<td>Autumn</td>
<td>stems</td>
<td>1525</td>
<td>905.8</td>
</tr>
</tbody>
</table>

aVegetation classification according to Acocks (1988)  
bSupplied by the South African Weather Bureau  
cMAR = mean annual rainfall
Dried milled samples were extracted one month after being harvested. The milled material was then stored away from direct light, in closed but not airtight containers, at room temperature (25–28°C) for a further eleven months before the second extractions were done.

Milled samples were boiled for an hour to mimic the methods used by traditional healers (± 100 g milled sample per litre double-distilled milli-Q water). The decoctions were settled overnight at 4°C. The supernatant was lyophilised then reconstituted at fixed concentrations in water.

Rat uterine tissue was obtained from oestrogenised female Spraque-Dawley rats (approximate weight 250 g), and mounted in Tyrode (physiological solution) at 26°C to decrease spontaneous contractions (Kumagai & et al. 1952). The organ baths were oxygenated with carbogen (5% CO2 in O2). Acetylcholine chloride (Sigma), a cholinergic agonist that stimulates contractions of smooth muscle was added cumulatively as the reference drug.

The effect of the plant extracts was tested by incubating the uterine tissue with 1.3 mg/ml crude plant for 5 minutes before the cumulative addition of acetylcholine, except for the control curve (+), where acetylcholine (ACh) only was added.

### Results

#### Distributional variation

The contractile activity of the aqueous extracts of *R. tridentata* harvested from different localities around South Africa varied greatly (Figure 1). Plants from all localities yielded extracts which had negligible effect on the maximal response of the organs to acetylcholine, except the extract from the plant harvested in Mondeor that halved the maximal response to acetylcholine. The initial response, which indicates the contractile response to the aqueous extract alone also varied. The extract harvested in Durban stimulated the largest contractions, where the samples from Umlazi and Mondeor yielded the least response. The extract from plant material stored for a month yielded a response identical to that from material stored for a year (Figure 2).

### Discussion

The results show there is a variation in the pharmacological response to aqueous extracts from *R. tridentata* plants harvested from different localities. All the extracts augmented the initial response of the organs to low dose acetylcholine, but the extent of augmentation varied. The effect on the maximal response is where the interesting difference occurred. The extract from the plant harvested in Mondeor (Figure 1) was the only extract to shift the dose response curve to the right and inhibit the response to maximal concentrations of acetylcholine, which suggests the extract is competing with acetylcholine for binding sites on cholinergic receptors. Most of the extracts from *R. tridentata* appeared to act as agonists (activate receptors) where the extract from Mondeor had results similar to those expected from an antagonist (block the effect of an agonist). This means different specimens of the same subspecies displayed opposite mechanisms of action. The contractions appear to be mediated through muscarinic receptors (Katsoulis et al. 1999b) which are present on most involuntary muscle, including the heart, lungs and intestines. In relation to the above results, one may postulate that extracts from one plant may cause contraction of smooth muscle, causing diarrhoea, where extracts from a plant from another region may cause smooth muscle relaxation leading to constipation. However, this would have to be confirmed in vivo.

The Mondeor extract seemed to compete with acetylcholine in a non-competitive manner. This inhibition of acetylcholine was negated by rinsing the bath inferring that the inhibition is reversible.

Extracts from *R. tridentata* roots were shown to have lower contractile activity than extracts from *R. tridentata* stems (Katsoulis et al. 1999b). The root extract from the plant harvested from Umlazi gave a response similar to that of extracts from stems harvested from Suikerbosrand (Figure 1). However, the roots harvested from Durban yielded an extract that stimulated contractions significantly higher than the contractions stimulated by the Suikerbosrand stem extracts.

Even though these results have illustrated the variation with distribution, no conclusions can be made about what factors may be responsible for this variation. Soil samples, the mean rainfall, temperature and the rate of photosynthesis prior to harvesting.
were not obtained. Further studies using controlled environments, such as in a phytotron, would be necessary to ascertain which factors influence the composition of compounds within the plant that are extracted in water.

The specimens tested did not exhibit much variation in gross morphology. The leaf shapes and sizes were very similar. The only difference in morphology was that the foliage of the specimen from Mondeor, which had antagonistic activity, was a lot more dense. Chemotaxonomic studies should be done to investigate whether the species delineation proposed by Urton (1986) is valid chemically. Perhaps the variation in these results is a manifestation of taxon differences which are not manifested in leaf morphology.

These results support the suggestion by Tobler (1994) that there should be a holistic approach to standardising the production of medicinal plants. The entire production process of medicinal plants should be controlled, starting from the cultivation and continuing the control through the processing of the plant material. Collecting the plants from "the wild" predisposes the patient to risks of toxicity through variation in the degree and type of response to the plant remedy. This distributional variation is compounded by the seasonal variation shown in Katsoulis et al. (1999a).

The results on the effect of storing dried plant material indicate that the active components of the plant that are extracted by water are stable at room temperature, and away from sunlight (Figure 2). This is relevant to the traditional healer as dried plant material is stored by medicinal plant vendors and by the traditional healers themselves. The results suggest that storing plant material does not alter the efficacy of the remedy.

**Acknowledgements**

B. Brookes and L. Harms for collecting plant material, and to Suikerbos Nature Reserve for allowing the author to harvest plant material.

**References**


The status of Larryleachia Plowes and additional synonymy for Lavrania Plowes

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Received 3 November 1998, revised 22 June 1999

The genus Larryleachia Plowes is shown to be superfluous and is reduced to synonymy under Lavrania Sect. Cactoidea. Full synonymy for the four species placed by Plowes in Larryleachia is given. Lavrania sect. Cactoidea is validated. A neotype is selected for Lavrania marlothii (N.E.Br.) Bruyns.

It is necessary to examine the ‘revision’ of Plowes (1996), where he proposed that the genus Lavrania (sensu Bruyns 1993) be split into two genera and the four species of Lavrania Sect. Cactoidea moved into a genus of their own (Larryleachia) with Lavrania once more monotypic (i.e. sensu Plowes 1986). It is particularly worrisome that this has been taken up uncritically in the recently published ‘List of southern African Succulents’ of the National Botanical Institute (Smith et al. 1997). As the generic arrangements within the stapelias are a cause for concern at present (see also Walker 1997), it is felt that the case of Larryleachia should be dealt with properly. I have, therefore, again examined the available material and revisited the arguments on which my concept of Lavrania was based and, if anything, this exercise has convinced me that my original treatment was correct and that Larryleachia is unnecessary.

I wish to examine the arguments put forward by Plowes (1996), where 12 ‘differences’ between Lavrania and Larryleachia were enumerated. Each of them will be considered in detail (throughout, the page numbers refer to Bruyns (1993) and ‘L.’ refers to Lavrania).

1. ‘In Larryleachia the flowers are borne in small groups of 1–3 that open successively... usually on young growth near the apex of the primary as well as other stems. In Lavrania the inflorescence is a fascicle of 6–15 flowers [which]... are not borne at the apex of the stems but low down near the base of new stems. Furthermore the primary stem does not bear flowers.’

In L. perlatia (p. 261) the flowers may be in groups of up to 12 per inflorescence. In the other three species, they arise in groups of 1–5 (6) per inflorescence (p. 246, 258). The number of flowers is, therefore, not diagnostic. In addition, in L. perlatia several flowers may be open at once on an inflorescence and the term ‘fascicle’ may be applied here too.

In the second place the position of the inflorescences is not as...