SEASONAL VARIATION IN UTEROTONIC ACTIVITY OF RHOICISSUS TRIDENTATA EXTRACTS

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Background. Rhoicissus tridentata lignotubers are widely used in southern African traditional pregnancy-related remedies.

Objectives. To determine the seasonal variation in contractile activity of extracts from different parts of the plant.

Methods. Isolated rat uterus tissue was used to compare the contractile activity of crude aqueous extracts of R. tridentata made from plant material harvested every 3 months over a period of 2 years.

Results and conclusions. The activity of the plant extracts from plants harvested in summer and autumn were 4 - 5-fold higher than extracts from plants harvested in winter or spring. The tubers stimulated the greatest degree of contractions, followed by the stems, roots and leaves. These results highlight the need to standardise the timing of harvesting R. tridentata.

METHODS

R. tridentata subsp. cuneifolia plant material was collected from Suikerbosrand Nature Reserve, 60 km south of Johannesburg. Three plants were harvested every 3 months from May 1996 to April 1998. The identity of the plants was validated by L Katsoulis, and one voucher specimen for each individual plant harvested was lodged in the C E Moss Herbarium (voucher specimen numbers of the three plants: 086928, 082929 and 082930).

Milled plant material was boiled for an hour, settled, and the supernatant lyophilised. Sections of uterine tissue obtained from the central portion of the uterine horns of oestrogenised virgin Sprague-Dawley rats were mounted in 50 ml organ baths containing aerated (5% CO₂ in O₂) Tyrode solution. The uterine tissue was maintained at 26°C to decrease spontaneous contractility. The organs were rinsed repeatedly while being allowed to return to baseline, before any investigations.

Control challenges with acetylcholine were done between each test challenge. The activity of the extracts was tested by incubating the tissues with 1.3 mg/ml of the various plant extracts for 5 minutes before adding acetylcholine cumulatively. This was done to determine whether the plant extracts alter the organ’s response to acetylcholine. Isotonic contractions were measured against 1 g resistance and expressed relative to the maximal response of the same tissues to acetylcholine. More details of the methods are given in the study by Veale et al.

RESULTS AND DISCUSSION

Fig. 1 illustrates how the different parts of the plants harvested in winter and spring all yielded aqueous extracts with low levels of contractile activity, whereas the extracts from different parts of the plants harvested during summer and autumn were considerably more active. During summer the tubers yielded the most active aqueous extracts, followed by the stems, roots and leaves. The autumn plants exhibited a similar pattern of depending on where the plant was harvested. Temporal variation is another possible source of variability in chemical constituents and biological activity of plants. This is a well-documented phenomenon. The expression of genes coding for chemical constituents of plants is under the control of various factors, such as the developmental stage of the organism, duration and intensity of light, nutrient supply, triggering of internal signals and sequential expression of genes coding for secondary metabolites. All the above factors vary temporally, which in turn causes seasonal fluctuations in the levels of both primary and secondary metabolites within plant tissues.

The aim of this study was to determine whether the contractile activity of R. tridentata varies seasonally.
• Tubers (N=6)
• Stems (N=15)
• Leaves (N=22)
• Roots (N=13)

+ Acetylcholine (N=16)

**Fig. 1.** Dose response curves of isolated rat uterus when pre-treated with 1.3 mg/ml aqueous extracts of all parts of *Rhoicissus tridentata* subsp. *cuneifolia* harvested during spring before the cumulative addition of acetylcholine. Each point represents the mean with the vertical bars representing the standard error of the mean (SEM).

Variation except that the roots were the least active part. The autumn and summer tubers were four and five times more active than the winter and spring extracts. The stems displayed similar seasonal variability.

Statistical differences between the direct contractile activity of the plants, shown by 'Rh' on the dose response curves, are given in Table I.

All parts of the plant harvested in summer and autumn were able to stimulate contractions of the isolated uterus directly. Traditional healers usually use the lignotubers, which yield the most uterotonic activity extracts. The activity of the tuber extracts is highest during the growing season and decreases during the seasons when the plant is dormant.

### Table I. Statistical differences between the contractile activity of extracts from different parts of *Rhoicissus* plants harvested during different seasons

<table>
<thead>
<tr>
<th>Tubers</th>
<th>Roots</th>
<th>Stems</th>
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<tbody>
<tr>
<td>Spring v. summer*</td>
<td>Spring v. summer*</td>
<td>Spring v. summer†</td>
</tr>
<tr>
<td>Spring v. autumn†</td>
<td>Summer v. autumn*</td>
<td>Autumn v. winter‡</td>
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*P < 0.01
†P < 0.001
‡P < 0.05

The root and stem extracts showed a similar pattern in seasonal variation in uterotonic activity to that of the tuber extracts. That is, the activity of the extracts started increasing when plants were harvested after the first rains. The activity increased throughout the growing season (or rainy season), reaching a peak in autumn. The activity of the extracts then fell as the plants lost their leaves and became dormant during winter. The rise in activity as the rains started could have been caused by the improved uptake of minerals from the soil following the mineralisation flush after the first rains, which usually leads to a sharp rise in the nitrogen content within the plant.

Variability in the contractile activity of the extracts could have been caused by: (i) altered concentrations of the active principle(s); (ii) a change in the quality or nature of the active principle(s); or (iii) changes in the production of compounds such as saponins which may have altered extraction of the pharmacologically active component(s).

**RELEVANCE TO CONSERVATION**

The stem extracts yielded a similar response to the most active part, namely the lignotubers. This is significant from an environmental perspective, as harvesting stems for the trade in traditional remedies would have a much lower impact on the
plant populations of R. tridentata than harvesting the lignotubers and roots. With current use of the lignotubers, the plant has to be uprooted for lignotubers to be harvested.

Clinical relevance

Variability in the uterine response to the decoctions from different seasons highlights a severe flaw in the use of traditional herbal remedies. Collectors and vendors have no way of determining the chemical composition of the plant material harvested. Eight traditional healers in and around Johannesberg were questioned on whether they were aware of any seasonal variation in the efficacy of R. tridentata. None of them seemed to be aware of the concept.

Traditional healers were also unaware of any toxic effects of the plant (personal communication) despite reports implicating the plant in fatal poisonings. This suggests that only certain plant samples may be toxic. It is possible that the toxicity of the plant also fluctuates seasonally, which would explain why traditional healers are generally unaware of the toxic potential of R. tridentata. Further investigation should be done to determine whether the poisonings that have occurred are concentrated at a certain time of the year.

Variability in the pharmacological activity of R. tridentata also reiterates the well-accepted importance of standardisation criteria that need to be established for herbal remedies. The results also support the suggestion by Tobler that there should be a holistic approach to standardising the production of medicinal plants by controlling the entire production process for medicinal plants, starting from cultivation and continuing the control through the processing of the plant material.

References


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