Cytotoxicity Screening of Endemic Plants from Roraima Tepui

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Study site

Mount Roraima

INTRODUCTION

• New strategies of research have proven an efficient and rapid tool for natural drug discovery. One of these, a **chemical ecology approach**, has been used to screen plants according to their plant growth habitat as an indicator of production of biologically active secondary metabolites. For instance, intense UV-B radiation in tropical mountains may induce production of these metabolites, affecting their composition profiles (1).

• **Tepuis** are isolated table mountains in Guayana Highlands that host a highly endemic flora of great interest in the search of new bioactive compounds. Plants inhabiting these plateaus are separated by the surrounding lowlands by vertical cliffs reaching altitudes from 1,500 to 2700 m, and must survive in an environment with limited nutrients supply and cope with extremes of radiation and temperature. This floristic province, called **Pantepui**, hosts some of the highest rates of plant endemism in northern South America (2). Of the 2,322 species of vascular plants in 630 genera, 766 are endemics to the province.

•. **Mount Roraima**, is the highest tepui in Guayana (2,723 m). Its tepuy summit hosts a genuine cloud habitat characterised by quaking bogs, rocky outcrops, and tangled vegetation. At 5° N latitude, environmental parameters are extreme and include harsh winds and bouts of intense rainfall followed by intense tropical sunshine. The highly endemic flora of this area and novel adaptations of its plants make them very interesting for screening studies of new phytochemical compounds of medical interest.

METHODS

Screening criteria

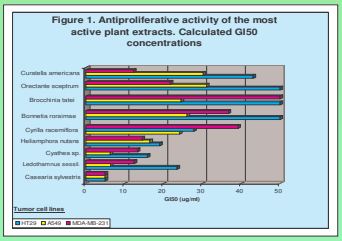
Leaf samples were high on priority collected from endemic species growing on the summit or at the rainforest that covers the slopes of Mount Roraima (Guyana):

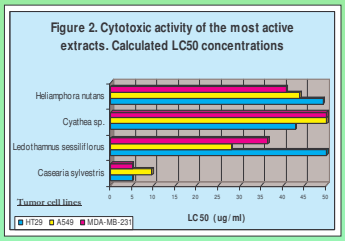
- **Plants endemic to Pantepui province**. *Drosera roraimae* (Droseraceae), *Epidendrum secundum* (Orchidaceae), *Heliamphora nutans* (Sarraceniaceae), *Sbefflera rugosum* (Araliaceae), *Stegolepis guianensis* (Rapataceae).
- **Plants endemic to Guayana region**: *Brocchinia tatei* and *B. reducta* (Bromeliaceae), *Orectante sceptrum* (Xirydaceae). *Ledothamnus sessiliflorus* (Ericaceae).
- **Other plants collected:** *Befaria imthurnii* (Ericaceae), *Bonnetia roraimae* (Theaceae), *Cyrilla racemiflora* (Cyrilliaceae), *Tillandsia turneri* (Bromeliaceae), *Cyathea sp.* (Cyatheaceae), *Casearia sylvestris* var. *lingua* (Flacourtiaceae), *Curatella americana* (Dilleniaceae). *C. sylvestris* (leaves) and *C. americana* (root) were selected due to medicinal uses indicated by our native guides.

Cytotoxicity assay *in vitro*

Extracts were obtained in isopropanol from dried leafs. A three tumor cell lines panel was used to determine dosage-dependent activity of every sample: **HT29 (colon), A-549 (lung) and MDA-MB-231 (breast).** Three extract concentrations (5, 15 and 50 µg/ml) were tested for every sample. **MTT** (tetrazolium blue) colorimetric assay was employed as test for quantification of cell proliferation and viability after 48-hour incubation (3). Sample effectiveness was assessed by two calculated response parameters: **GI50** to determine the concentration for 50% growth inhibition, and **LC50** for 50% tumor killing activity. These are interpolated values from the dose-response curves. In case these parameters could not be determined by interpolation of the data, "default values" were assigned, corresponding to the highest (50 µg/ml) or lowest (5 µg/ml) tested concentration, according to the NCI in vitro anticancer screen (4). Only samples showing less than 32% net growth in at least one cell line, in the range of concentrations tested, or more than 50% cell killing, were considered for GI50s or LC50s determination, respectively.

RESULTS





Growth inhibition activity

• Extracts of nine species caused less than 32% growth in at least one cell line. Estimated concentrations resulting in 50% growth inhibition (GI50) for these species against the three cell lines are shown in Fig. 1.

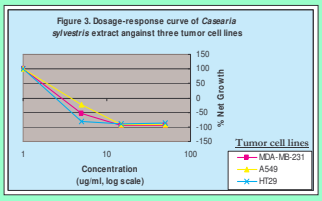
• The highest inhibition activities (GI50s<25 µg/ml) were shown by the four species that also had highest cytotoxic activity (Fig. 2). Antiproliferative activity of these samples was mostly exhibited against lung tumor cells, with GI50s of 5 µg/ml for ***C. sylvestris***, 6.4 µg/ml for ***L. sessiliflorus***, and 6.3 µg/ml for the tree fern ***Cyathea sp.***, the most active extracts. These four species, were also the most active against breast cells, ranging from <5 µg/ml in ***C. sylvestris*** to 14.5 µg/ml in ***H. nutans***; also against colon cells, with GI50s ranging from <5 µg/ml for ***C. sylvestris*** to 23.5 µg/ml for ***L. sessiliflorus***.

• ***C. sylvestris*** was by far the most active antitumor extract, with GI50s below the lowest concentrations tested against the three cell lines (<5 µg/ml).

Cytotoxic activity

• Significant cytotoxic activity (LC50<50 µg/ml in at least one cell line) was detected in four species (Fig. 2). ***L. sessiliflorus*** extract showed medium cytotoxic activity against lung and breast lines (LC50s of 27.9 and 36.3 µg/ml, respectively); medium activity was also found in ***Cyathea sp*** against colon (LC50 of 42.7 µg/ml) and *H. minor* against breast and lung cells (LC50s of 40.6 and 43.7 µg/ml respectively). Other species, ***C. racemiflora***, ***C. americana*** and ***B. roraimae***, showed lower but significant cytotoxicity at 50 µg/ml dose, with LC50s >50 µg/ml (data not shown).

• The most cytotoxic plant against the three cell lines was ***Casearia sylvestris*** (Fig. 3), with the highest activity (81% cell killing) against colon cells, at the lowest concentration tested (5 µg/ml), and a LC50 value below this concentration. This species was also the most cytotoxic against lung cells (24% cell killing at 5 µg/ml, LC50 of 8.3 µg/ml) and breast cells (53% cell killing at 5 µg/ml, LC50 <5 µg/ml). At higher concentrations (15 and 50 µg/ml), no significant differences in cytotoxic activity against the three lines was shown by this plant.



Casearia sylvestris

DISCUSSION

Screening conclusions

• The nine samples which reduced net growth of at least one of the cell lines tested to less than 32% can be considered worthy of further study (Fig. 1). Although three lines pre-screen has been shown to efficiently remove many of the inactive samples from unnecessary and costly full scale evaluation (5), a broader concentration range, and more complete cell panel assay, such as NCI's 60-cell line assay (6), would be the next advisable step in order to confirm and characterise the antiproliferative activities detected in these samples. According to our pre-screening data this condition was fulfilled in over half (9/16) of the species tested (Fig 1). Additionally, one quarter of total species showed also significant tumor killing activity (Fig. 2).

Active agents

• New cytotoxic clerodane diterpenes, named casearins, have already been isolated and described from ***Casearia sylvestris***, a medicinal plant (7). Research on casearins is ongoing (8), and might be able to develop these chemicals into new effective chemotherapeutic agents in the future. However, the variety *lingua* collected here had never been essayed for cytotoxicity. Some other known antitumor agents, such as sesquiterpenes α-humulene, β-elemene, β-caryophyllene were detected by our group in preliminary GC-MS carried on this sample (data not shown). The relative influence of these chemicals, as well as casearins, in the activity detected is undetermined until now.

• Some known cytotoxic agents, such as triterpenoids and xanthones**,** have already been isolated from other species of the genera ***Cyathea*** (9) and ***Bonnetia*** (10), respectively. However, although these or related compounds might be responsible for part, if not all of the activity detected in the samples collected in Roraima, at the moment we have no concluding evidences supporting this assumption. No cytotoxic compounds have been reported from the three other species with cytotoxic activity cited in this work: ***L. sessiliflorus, H. nutans*** and ***C. racemiflora.***

• Little is known about the active compounds or possible synergistic effects that might be responsible for the measured activity in these plants. Some novel active chemicals yet to be discovered might be responsible for cytotoxicity in some extracts. On the other hand, characterisations of secondary metabolites profiles in response to UV-B radiation are rare and might be the key to some of cytotoxic activities detected (1). Unfortunately, we have not had the resources, nor the amount of samples needed to complete the isolation and identification of these agents by cytotoxicity-guided fractionation.

Ledothamnus sessiliflorus

Cyathea sp.

Heliamphora nutans

Future research

• The most promising species for further research are ***C. sylvestris***, ***Cyathea sp.***, ***L. sessiliforus*** and ***H. nutans***. Cytotoxicity-directed fractionation of these extracts would be then advisable in order to isolate and identify the main active principles involved. Once the in vitro cell growth patterns for these compounds are determined in 60-cell panel, antitumor mechanisms of action might be characterized, or possible unknown mechanisms detected, by means of the COMPARE pattern-recognition algorithm (11).

• The most potent cytotoxic extract, ***C. sylvestris* var. *lingua***, deserves to be retested in a broader cell lines panel, and lower concentration ranges to determine more accurate LC50s and GI50s values than the default values shown in this study.

• Nevertheless, in order to continue research, collections of higher, even massive, amounts of plant material would need to be collected for the next steps, and this can be a handicap as some of these are endemics and highly protected species, growing in specific radiation and other difficult tropical mountains conditions, so that the potential for mass cultivation of the plants of interest would need to be assessed.

CONCLUSIONS

✓ Nine out of sixteen plants collected at Mount Roraima showed significant tumor growth inhibition activity. Four of them presented in addition significant cytotoxic activity

✓*Casearia sylvestris* var. *lingua* showed the highest antitumor activity among all plants tested and against all cell lines

✓ Endemic plants inhabiting Guayana Tepuis can be considered as promising candidates for cytotoxicity screening programs. Further phytochemical research and better conservation strategies of this truly unique and pristine ecosystem are worthy to be promoted

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