# THE ECOLOGY OF AN ELFIN FOREST IN PUERTO RICO, 13. PHYTOCHEMICAL SCREENING AND LITERATURE SURVEY

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AS PART OF A DETAILED STUDY on the restricted flora of the elfin forest on the summit of Pico del Oeste, thirty-five species of plants were screened for the presence of three phytochemical constituents, alkaloids, saponins, and tannins. The literature was reviewed to determine which of these constituents had previously been found in the species cited or in related ones.

## MATERIAL AND METHODS

PREPARATION OF EXTRACTS: The screening procedures for alkaloids, saponins, and tannins were adapted from those described by Wall *et al.* (1, 2). An extract of each plant sample was prepared by refluxing 50 grams of the air-dried, milled plant material with 300 ml. of 80 percent ethanol for one hour. Each extract was then cooled to room temperature, filtered by suction, and the residue was washed with sufficient 80 percent ethanol to bring the volume of filtrate to 500 ml.

**Alkaloids.** Fifty ml. of each extract was evaporated to dryness using a steam bath after which the residue was stirred with five ml. of 1 percent aqueous hydrochloric acid. One ml. of the filtrate was treated with a few drops of Mayer's reagent and a separate one ml. portion was treated similarly with silicotungstic acid reagent (12 percent aqueous). Precipitation or turbidity with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated. A confirmatory test, designed to remove non-alkaloidal compounds capable of eliciting "false-positive" reactions with either of these reagents was conducted in the following manner with all extracts giving a preliminary positive test for alkaloids.

Two ml. of the acidic aqueous extract, prepared as described above, was treated with 28 percent ammonium hydroxide solution until the solution was distinctly alkaline to litmus paper, and then extracted several times with chloroform. The chloroform extracts were combined and concentrated *in vacuo* to *ca.* two ml., then extracted with an equal volume of 1 percent aqueous hydrochloric acid. One ml. of the separated acid extract was treated with a few drops of Mayer's reagent, and a second one ml. portion was treated with silicotungstic acid reagent as previously described. Turbidity or precipitation after the addition of either of these reagents was taken as a confirmed positive test for the presence of alkaloids in the extract. The results of these tests are presented in TABLE 1.

Each plant sample was also screened for alkaloids using thin-layer chromatography according to the method of Farnsworth and Euler (3). This procedure was modified only in that the final volume of fraction I (chloroform extract) applied to each thin-layer plate was 30 $\mu$l. The results from this test are also presented in TABLE 1.

**Saponins.** Since all saponins, whether steroidal or triterpenoid, will hemolyze red blood cells, utilization of this property is advantageous for detecting this class of compounds in plant material. A red blood cell suspension was prepared and standardized against digitonin according to the protocol of Wall *et al.* (2). One ml. of each plant extract was mixed with 10 ml. of the red blood suspension and the mixtures were allowed to stand for one hour before observing the results. Complete hemolysis of the red blood cells in any instance was taken as evidence for a positive test, the results of which are presented in TABLE 1.

**Tannins.** 100 ml. of the original 80 percent ethanol extract from each plant sample was evaporated to dryness on a steam bath and the residue was stirred with 25 ml. of distilled water and filtered. Two ml. of the filtrate was treated with a few drops of gelatin-salt reagent (1). Precipitation along with a color reaction following the addition of ferric chloride reagent (blue-black, black, green-black) was positive evidence for the presence of tannins, green-black indicating tannins of the catechol type and black indicating tannins of the pyrogallol type. No precipitation with the gelatin-salt reagent and a green color with ferric chloride indicated the presence of phenolic compounds but not necessarily tannins. Precipitation with the gelatin-salt reagent and a brown color with ferric chloride indicated no tannins present.

## SUMMARY

Of the thirty-five species of plants tested, alkaloids alone were found in two, alkaloids and tannins in three, saponins alone in one, saponins plus tannins in one, and tannis alone in thirteen. The remaining fifteen species contained none of these compounds although at least three gave tests for phenolic compounds.

With the exceptions of a report of alkaloids on *Lobelia portoricensis* and earlier work on *Cecropia peltata*, no published phytochemical data for any of the species of the test site were found. Our findings of alkaloids in *Hillia parasitica* and *Psychotria berteriana* are consistent with earlier reports of the presence of these constituents in other species of the same genera. On the other hand, our sample of *Ilex sintenisii* gave no evidence for the presence of caffeine and tannin for which *I. paraguariensis* is well-known. Alkaloids found in other species of *Justicia*, and the alkaloids, saponin, and tannin reported for other species of *Carex*, were not found in *Justicia martinsoniana* or *Carex polystachya* respectively.

TABLE 1. **Results of Phytochemical Screening**

[Literature references are to reports of various constituents for these or related species]

| SCIENTIFIC NAME | PART[a] USED | ALKALOIDS[b] M[c] | ALKALOIDS[b] S[d] | ALKALOIDS[e] Fraction I | ALKALOIDS[e] Fraction II | TANNINS Gelatin salt | TANNINS $FeCl_3$[f] | SAPONINS | LIT. REF. |
|---|---|---|---|---|---|---|---|---|---|
| ACANTHACEAE | | | | | | | | | |
| *Justicia martinsoniana* | wp | – | – | – | – | – | – | – | (6–12) |
| AQUIFOLIACEAE | | | | | | | | | |
| *Ilex sintenisii* | st/lf | – | – | – | – | + | br | – | (13–34) |
| BIGNONIACEAE | | | | | | | | | |
| *Tabebuia rigida* | st/lf | – | – | – | – | + | gr/bk | – | (35–43) |
| BROMELIACEAE | | | | | | | | | |
| *Vriesea sintenisii* | wp | – | – | – | – | – | gr | – | |
| CAMPANULACEAE | | | | | | | | | |
| *Lobelia portoricensis* | st/lf | + | + | + | + | – | gr | – | (44–54) |
| CELASTRACEAE | | | | | | | | | |
| *Torralbasia cuneifolia* | st/lf | – | – | – | – | + | br | – | |
| CYPERACEAE | | | | | | | | | |
| *Carex polystachya* | st/lf | – | – | – | – | – | br | – | (55–62) |
| *Eleocharis yunquensis* | wp | – | – | – | – | – | – | – | (63) |
| *Scleria secans* | wp | – | – | – | – | – | br | – | |
| CYRILLACEAE | | | | | | | | | |
| *Cyrilla racemiflora* | st/lf | – | – | – | – | + | bk | – | |
| ERICACEAE | | | | | | | | | |
| *Hornemannia racemosa* | st/lf | – | – | – | – | + | gr/bk | – | |
| GESNERIACEAE | | | | | | | | | |
| *Gesneria sintenisii* | st/lf | – | – | – | – | + | br | – | (64) |
| GRAMINEAE | | | | | | | | | |
| *Arthrostylidium sarmentosum* | st/lf | + | + | – | – | – | gr | – | |
| GUTTIFERAE | | | | | | | | | |
| *Clusia grisebachiana* | st/lf | – | – | – | – | + | bk | – | (65) |
| LAURACEAE | | | | | | | | | |
| *Ocotea spathulata* | wp | – | – | – | – | + | br | – | (66–72) |
| MARCGRAVIACEAE | | | | | | | | | |
| *Marcgravia sintenisii* | st/lf | – | – | – | – | + | br | – | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MELASTOMATACEAE | | | | | | | | | |
| *Calycogonium squamulosum* | st/lf | − | − | − | − | + | gr/bk | − | |
| *Mecranium amygdalinum* | st/lf | − | − | − | − | + | gr/bk | − | |
| *Miconia foveolata* | st/lf | − | − | − | − | + | gr/bk | − | |
| *Miconia pycnoneura* | st/lf | − | − | − | − | + | br | − | |
| MELIACEAE | | | | | | | | | |
| *Trichilia pallida* | st/lf | + | + | + | − | + | bk | − | (77) |
| MORACEAE | | | | | | | | | |
| *Cecropia peltata* | lf | − | − | − | − | + | br | − | (78,79) |
| MYRSINACEAE | | | | | | | | | |
| *Ardisia luquillensis* | st/lf | − | − | − | − | + | bk | − | (80–84) |
| MYRTACEAE | | | | | | | | | |
| *Calyptranthes krugii* | st/lf | − | − | − | − | + | br | − | |
| *Eugenia borinquensis* | st/lf | − | − | − | − | + | bk | − | (85–90) |
| OLEACEAE | | | | | | | | | |
| *Haenianthus salicifolius* var. *obovatus* | st/lf | − | − | − | − | + | bk | − | |
| RUBIACEAE | | | | | | | | | |
| *Hillia parasitica* | st/lf | + | + | + | + | − | − | − | (91) |
| *Psychotria berteriana* | st/lf | + | + | + | − | + | gr/bk | + | (92–95) |
| *Rondeletia portoricensis* | st/lf | − | − | − | − | + | bk | − | (96) |
| SAPOTACEAE | | | | | | | | | |
| *Micropholis garciniaefolia* | st/lf | − | − | − | − | + | bk | − | |
| SYMPLOCACEAE | | | | | | | | | |
| *Symplocos micrantha* | st/lf | − | − | − | − | + | br | − | (97–100) |
| THEACEAE | | | | | | | | | |
| *Cleyera albopunctata* | st/lf | − | − | − | − | + | gr/bk | − | |
| THYMELAEACEAE | | | | | | | | | |
| *Daphnopsis philippiana* | st/lf | + | + | − | + | + | gr/bk | − | |
| URTICACEAE | | | | | | | | | |
| *Pilea yunquensis* | wp | − | − | − | − | − | − | − | (101–103) |
| ZINGIBERACEAE | | | | | | | | | |
| *Renealmia antillarum* | wp | − | − | − | − | + | − | + | |

Key: [a]wp = whole plant, st/lf = stems and leaves; [b]Wall *et al.* procedure (1), based on confirmatory test; [c]Mayer's reagent (4); [d]Silicotungstic acid (5); [e]Farnsworth and Euler procedure (3); [f]br = brown, gr/bk = green-black, bk = black, gr = green.

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