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Exhaustive extraction of phenolics and tannins from some sun-exposed forbs and shrubs of the tropical Andes

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Abstract

Monomeric and polymeric phenolic derivatives have attracted renewed attention due to novel discoveries and applications in the pharmaceutical and food industries. Although lower and higher plants generally synthesize these compounds as allomones, antioxidants, ultraviolet (UV) radiation filters, and for other purposes, relatively few species accumulate them in sufficient quantity to be of interest for industrial applications. For the most part these plants are woody perennials with long-lived leaves. Assuming that the metabolic investment of plants growing in high elevation regions is greater than that of lower areas due to the need to protect sun-exposed tissue to excess UV-B radiation and avoid the associated damage to DNA and free radical-mediated redox processes, we anticipated to find useful amounts of these materials in Andean forbs of low-mid stature and limited leaf longevity in western Venezuela where the UV-B contribution to solar radiation is high. Therefore the aims of this investigation were: 1) Select a group of representative Andean species of mid altitude flora in sun-exposed meadows. 2) Devise appropriate extraction methods to obtain the highest possible yield of phenolic and condensed tannins. 3) Compare the response of phenolic material during extraction depending on plant species. Eight plant species (*Alnus acuminata, Clidemia ciliata, C. flexuosa, C. hirta, Miconia tuberculata, Monochaetum meridensis,* and *Psamisia penducifolia*) of three distantly related dicotiledoneous families (Betulaceae, Ericaceae, Melastomataceae) and a fern (*Pteridium arachnoideum*) (Dennstaedtiaceae) growing at 2200 m above sea level and commonly found across the Andes were selected for study during the rainy season. Repeated sequential treatment of vacuum dried plant leaves and blending in 70% aqueous acetone at room temperature followed by sonication at 4-6°C was employed to obtain the phenolic-condensed tannin enriched extract. The monomeric and polymeric fractions were separated by exclusion chromatography and quantified by the modified Prussian Blue specrophotometric method. These species were found to contain elevated levels of phenolics (30-123 mg salicylic acid eq. g^{-1} dw of leaf) and tannins (20-521 mg quebracho tannin eq g^{-1} dw of leaf) and to require between four and five consecutive extractions before a negative Prussian blue test of the extracted material could be attained. Single solvent treatments yielded only 21.1-80.3% of monomeric phenolics and 25.1-84.8% of condensed tannins, depending on plant species, although time of extraction did not improve yields. However, better yields were obtained by raising the volume/sample weight ratio from 70/5 (mL/g) to 140/5 although no further increments could be procured at higher

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ratios presumably because of inefficient blending of leaf particles suspended in the solvent. Surprisingly, *C. flexuosa* and *M. meridensis* furnished a higher yield of phenolics and tannins in the second extraction batch than in the first. To the competitive contribution of other more soluble solutes that are discharged in the first batch was attributed this odd behavior. We conclude that 1) plants growing in open areas at mid altitude in the tropical Andes contain a large proportion of phenolic/condensed tannin material. 2) These compounds need at least three and occasionally more sequential extractions for adequate removal from plant tissue and is strongly species dependent. 3) Solubility in aqueous acetone, adequate particle blending and competition by other components in the plant all contribute to extraction efficiency. 4) The high contents of phenolics and tannins found for the first time in some of these fast growing plants opens the possibility of their exploitation as new sources of these compounds.

Key words: Andes; Betulaceae; condensed tannins; Dennstaedtiaceae; Ericaceae; extraction; Melastomataceae; neotropics; phenolics; Venezuela.

Extracción exaustiva de fenólicos y taninos de algunas especies herbáceas y arbustivas de los Andes tropicales

Resumen

Existe un renovado interés por los derivados fenólicos tanto monoméricos como poliméricos debido a nuevas aplicaciones en las industrias farmacéutica y de alimentos. Aunque las plantas inferiores y superiores sintetizan estos compuestos en forma generalizada para su acción como alomonas, antioxidantes, filtros ultravioleta (UV) y otros fines, pocas especies relativamente los acumulan en cantidad suficiente para ser de interés en la industria. En la mayoría de los casos estas con perennes leñosas con hojas longevas. Bajo la suposición de que la inversión metabólica de plantas que crecen en regiones de altura es mayor que en las de zonas bajas, debido a la necesidad de proteger tejido expuesto a exceso de UV-B y evitar así daño al ADN y a procesos de degradación redox de tipo radical libre, anticipamos hallar cantidades útiles de estas sustancias en herbáceas de estatura entre baja e intermedia de hojas de longevidad limitada en los Andes del oeste venezolano en los que la contribución del UV-B a la radiación solar es elevada. Por lo tanto los objetivos de esta investigación fueron: 1) Seleccionar un grupo de especies representativas de la flora andina de altitud intermedia en herbazales expuestos. 2) Poner a punto un método de extracción adecuado para obtener el mayor rendimiento de derivados fenólicos y taninos condensados. 3) Comparar la respuesta del material fenólico extraído según la especie. En un prado expuesto a 2200 m sobre el nivel del mar se seleccionaron ocho especies vegetales (*Alnus acuminata, Clidemia ciliata, C. flexuosa, C. hirta, Miconia tuberculata, Monochaetum meridensis*,y *Psamisia penducifolia*) de tres familias distantes de dicotiledóneas (Betulaceae, Ericaceae, Melastomataceae) y un helecho (*Pteridium arachnoideum*) (Dennstaedtiaceae) en la época lluviosa. Tratamiento secuencial repetido de las hojas secadas al vacío por licuado en acetona acuosa al 70% a temperatura ambiente seguida de sonicación a 4-6°C produjo un extracto enriquecido en fenólicos y taninos condensados. Se separaron las fracciones monomérica y polimérica mediante cromatografía de exclusión y se cuantificaron mediante el método espectrofotométrico modificado de Azul de Prusia. Las especies estudiadas contuvieron niveles elevados de fenólicos monoméricos (30-123 mg eq. de ácido salicílico g^{-1} de peso seco

(ps) de hoja) y taninos condensados (20-521 mg eq. de tanino de quebracho g^{-1} (ps) de hoja) y requerir entre cuatro y cinco extracciones consecutivas antes de alcanzar una prueba negativa del análisis de Azul de Prusia. La extracción con un solo lote de solvente produjo entre 21,1 y 80,3% de fenólicos monoméricos y de 25,1 a 84,8% de taninos condensados según la especie, aunque tiempos más largos de extracción no mejoraron los rendimientos. Sin embargo, estos fueron mayores al elevar la relación volumen de solvente/peso del material vegetal de 70/5 (mL g^{-1}) a 140/5 y el aumento de esta relación no reportó mejoría en el rendimiento debido al licuado ineficiente de las partículas foliares suspendidas en el solvente. Resultó inesperado que *C. flexuosa* y *M. meridensis* produjeran mayores rendimientos de los dos grupos de sustancias en la segunda extracción secuencial que en la primera. Este resultado se atribuyó a la contribución competitiva de otras sustancias solubles presentes en las hojas que resultaron extraídas en mayor proporción en el primer lote. Concluimos que: 1) Las plantas que se desarrollan en áreas expuestas de altitud intermedia de los Andes tropicales contienen una cantidad importante de material fenólico y taninos condensados. 2) Para una extracción efectiva estos compuestos requieren de al menos tres y más tratamientos secuenciales con solvente y su número depende de la especie. 3) La solubilidad en acetona acuosa, el licuado apropiado y la competencia por otros compuestos presentes en la planta afectan la eficiencia de la extracción. 4) El elevado contenido de fenólicos monoméricos y taninos condensados hallados en algunas de estas plantas de rápido crecimiento abre la posibilidad de su explotación comercial para producir estas sustancias.

Palabras clave: Andes; Betulaceae; Dennstaedtiaceae; derivados fenólicos; Ericaceae; extracción; Melastomataceae; neotrópicos; taninos condensados; Venezuela.

Introduction

Phenolics and tannins are compounds of ample distribution in lower and higher plants which are derived from the shikimate and phenyl propanoid biosynthetic pathways (1, 2). Phenolics generally are monomers of low molecular weight whereas tannins are a group of polar olygomeric or polymeric phenolic derivatives in the 500 to 30,000 Dalton range. Many accumulate in marine and terrestrial plants with possible adaptation roles such as carbon sinks, feeding deterrents, digestion inhibitors, allelopathic materials, and solar radiation screens (3-5). Their protein-binding properties (6) have found practical use in leather processing, wound healing, bleeding control, disinfection and other applications, but this very reactivity constitutes a serious problem for the animal feed industry (7, 8). Their chemistry, role in ecology and industrial applications all have been reviewed extensively (1, 9, 10).

Phenolics have attracted the attention of food and health scientists in recent times due to their antioxidant properties and uses in health care (11) as for example effective antioxidants (12), efficient inhibitors of the proliferation of human T-cells (13) and human colon adenocarcinoma Caco2 cell lines (12), controllers of plasma apolipoproteins (14) and several others.

Plants in general tend to accumulate more phenolic materials when exposed to high levels of solar radiation, either naturally (15-19) or under glasshouse conditions (20), not only in vascular plants but in lichens as well (21). This has been attributed in part to the strong UV filtering capacity of the aromatic moiety of phenolics (22-24) and, this is sufficient to exclude most of the damaging UV (280-320 nm) radiation reaching th earth surface (25). Other environmental constraints also modify phenolics synthesis. Hence, the particular composition of the phenolics mixture varies consid-

erably depending on plant part, season, environmental or biological stress (15, 18, 26-29) and rain regime (30-32).

Heliophytes growing at high altitude in tropical mountains are naturally exposed to high levels of UV-B radiation (33). Plants are known to respond to altitudinal gradients by accumulation of leaf phenolics (19, 34). This is due to specific photoreceptors in leaves which regulate the formation of UV-B shielding pigments (35). It is mostly in woody perennials with long lasting leaves where these compounds occur in larger quantity but their economic exploitation may be hampered by their slow growth. Therefore, it was deemed of interest to determine the phenolics content of various typical plant species of the exposed herbal and shrub strata of relatively fast growth in a plant community around the species-diverse 2000 m altitudinal level in the central Andes mountains of Mérida State in Venezuela with the long term purpose of exploiting these plants as useful sources of phenolics and tannins and also to contribute to understanding the role played by these compounds in the antiherbivore defense and intraplant competition in these densely populated habitats.

To this end, we pursued specifically to determine the extraction conditions under which the greatest amount of monomeric phenolics and tannins, separately, could be obtained from plant tissue. This was put to test for eight plant taxa, which occur frequently in these mid altitude habitats.

Materials and Methods

Reagents

Potassium ferrocyanide was purchased from Aldrich Chemical Co, Milwaukee, Wisconsin, and ammonium ferric sulfate, salicylic acid and lipophilic Sephadex LH-20 were obtained from Sigma Chemical Co., St. Louis, Missouri. Quebracho tannin was kindly supplied by professor Ann Hagerman and purified by exclusion chromatography following her instructions (personal communication); 12 N hydrochloric acid and organic solvents were acquired from Riedel de Haen AG, Germany and water was distilled from an all-glass apparatus.

Plants

The selected species were *Alnus acuminata* Kunth (Dicotyledoneae, Betulaceae), *Clidemia ciliata* D. Don, *Clidemia flexuosa* Triana, *Clidemia hirta* D. Don, *Monochaetum meridensis* Karst, *Miconia tuberculata* (Naud.) Triana, (all of the latter Melastomataceae, Dicotyledoneae), *Psamisia penducifolia* (Dicotyledoneae, Ericaceae), and *Pteridium arachnoideum*. L. Maxon (Pteridophyte, Dennstaedtiaceae). Leaves of the above species were collected from a complex and diverse heliophyte community in Cerro La Bandera, Mérida, at 2200 m above sea level –asl–, and the vicinal Valle del Mucujún at 2400 m asl, (see below for description) during the rainy season in July, October and December 2000. Voucher specimens were kept in the Herbarium of Jardín Botánico, Universidad de Los Andes, Mérida and in our laboratory, and each species was determined by taxonomist Giuseppe Adamo of this institution.

Collection site

Cerro La Bandera is located in the north end of the city of Mérida, 8° 38´N, 71 09´W. At mid altitude (2200 masl) this mountain flattens to an open extensive meadow frequently affected by wildfires in the dry season (January to mid March). The rainy season extends from the second half of March to mid December (1800 mm rain). Temperatures are moderate with high variations between day and night (avg: 16°C, max.: 32°C, min: 6°C). These conditions give rise to the growth of certain predominant species whose composition changes considerably as the succession matures. Among the selected traits there are: resistance to fire, short development time or considerable

underground growth favoring rhizomatous or bulbous plants, or those with a strong capacity to colonize new land by abundant seed production and wind dispersal. Among the predominant species are elements of the Asteraceae, Dennstaedtiaceae, Ericaceae, Melastomataceae, Liliaceae, Orchidaceae, Poaceae, and some Pteridophytes including various fern and lycopodium species. Besides the low stratum, composed by small ferns and Poaceae, the rest of the vegetation remains with active foliage in the dry season. A moderate inclination of the terrain (5%) prevents the occurrence of soggy soils and bogs.

Extraction procedure

Freshly excised leaves of each species were vacuum dried at 0.2 mm Hg (36, 37) for 24 h and stored frozen for a few days before use. This method has been qualified as furnishing maximum yields of phenolics also by other authors recently (38). An exact weight of sample (2-11 g depending on content of phenolic/tannin material) was blended at high speed in aq acetone (70%, 70 mL) containing ascorbic acid (0.01%) as antioxidant, at room temperature for 30 min, followed by sonication (Elma Transsonic-310, Germany) for an additional 30 min in an ice-water bath (4°C). The suspension was centrifuged at 3000 rpm for 10 min and the acetone in the supernatant was evaporated under reduced pressure below 30°C. The volume of the remaining aqueous solution was brought to 100.0 mL from which a 2.0 mL aliquot was drawn. As the aqueous acetone also brings into solution many propenylphenolic compounds that may contribute to the color test, extracts were chromatographed through hydrophilic Sephadex (LH-20) to discriminate between these monomers and the polymeric tannin material. Elution with methanol (90%; 90 mL) yielded the monomeric phenolics fraction and further elution with aqueous acetone (70%; 90 mL) furnished the condensed tannins. Volumes given here are average but could vary according to the amount of phenolics and tannins present in the plant. Organic solvents in each fraction were evaporated in vaccuo and subjected to the oxidative assay described below. A minute sample of the solid pellet from the above centrifugation was observed under the microscope (x100) and found to be constituted of well ruptured plant tissue. However, addition of a droplet of ammonium ferric sulfate followed by another of potassium ferrocyanide in hydrochloric acid developed into a deep blue color that exposed the presence of residual phenolic material still embedded within fragmented leaf tissue. Therefore, the pellet was re-suspended in a fresh batch of acetone (70%; 70 mL) and the extraction procedure was repeated until no further reaction to the Prussian blue test could be observed in the supernatant or the mull under the microscope.

Monochaetum meridensis was also processed as follows: five batches (5 g each) of vacuum-dried leaves for 72 h were blended at high speed for 30 min and left in contact with the solvent during various time lengths before sonication and centrifugation: Sample 1: 30 min. Sample 2: 24 h. Sample 3: three days. Sample 4: five days. Organic solvents from the extracts were removed by evaporation under 30°C and total phenolic content was determined by the modified Prussian Blue method. Additionally, two separate batches of *M. meridensis* blended leaves (5 g) were extracted using 70 and 140 mL aqueous acetone, respectively, until complete exhaustion of Prussian Blue-reactive compounds, and total phenolics were determined as above.

Quantitation of tannins and phenolics

To derive calibration curves, quebracho (*Schinopsis quebracho-colorado*) (39) tannin was used as standard for tannin quantitation and was purified by chromatography through lipophillic Sephadex LH20 (40, 41) whereas salicylic acid was used as standard for monomeric phenolics.

Both purified compounds were subject to treatment with ammonium ferric sulfate and potassium ferric cyanide in mineral acid and their respective absorbances at 550 nm recorded at various concentrations. Thus, total content of tannins was expressed as mg of purified quebracho tannin equivalents per g of plant dry weight and total monomeric phenolics were reported as mg of salicylic acid equivalents per g of plant dry weight.

A modification of the Prussian blue method was employed for the quantifications (42). To 1.0 mL of the test solution stemming from the corresponding phenolics / tannin Sephadex chromatography, in a 125 mL ehrlenmeyer flask was added 50.0 mL of distilled water, and 3.0 mL of 0.1 M aqueous ammonium ferric sulfate in 1.0 mL portions and 1 min intervals with continuous stirring. After exactly 20 min, 3.0 mL of 0.008 M potassium ferric cyanide in 0.1 M aqueous hydrochloric acid was added also in 1.0 mL portions per min and vigorous stirring. After an additional 20 min, the absorbance at 550 nm was obtained with the aid of a Hewlett Packard Vectra UV-Vis spectrophotometer and a Vectra-Pentium workstation, using 5 mL, 1 cm optical path quartz cells.

Results

All studied species possessed a high content of aqueous acetone-soluble phenolic compounds (Table 1) and condensed tannins (Table 2) and required several sequential extraction steps before exhaustion of these materials from the leaf matrix. This was species dependent. Although there was a parallel extraction response of phenolics and condensed tannins, there were also anomalies as shown by Figure 1 for *A. acuminata.* However, the general trend was towards exhaustion of the source which responded differently depending on the v/w ratio of solvent/leaf material used (Figure 2). Extraction yields were independent of exposure time as illustrated by treatment of *M. meridensis* (Table 3).

Discussion

Alnus acuminata, a relatively common tree of the temperate riparian forests in the northern Andes and Central America, and it was selected as a model because of its high

Table 1
Yields (% of total recovered) of simple phenolics during sequential extractions of freeze-dried leaves of various phenolic rich Andean plant species. SD = one standard deviation

Species	Phenolics % of total					Total phenolics mg eq (*)	SD
	Ext 1	Ext 2	Ext 3	Ext 4	Ext 5		
Alnus acuminata	21.1	20.4	18.4	8.3	ND	44.6	3.9
Clidemia ciliata	80.3	15.4	3.3	1.0	ND	58.2	14.1
Clidemia flexuosa	21.8	75.2	1.6	1.4	ND	123.8	9.1
Clidemia hirta	58.3	21.0	14.2	6.6	ND	119.7	12.4
Miconia tuberculata	40.8	39.8	8.8	10.5	ND	30.3	2.8
Monochaetum meridensis	26.4	55.4	15.0	3.2	ND	104.2	12.5
Psamisia penducifolia	38.7	36.8	14.4	10.1	ND	103.1	13.0
Pteridium arachnoideum	82.4	16.2	1.4	ND	-	25.0	2.2

(*) Eequiv of salicylic acid per g of dry leaf.

Table 2
Yields (% of total recovered) of condensed tannins during sequential extractions of freeze-dried leaves from studied species

Species	C. Tannins % of total					Total C. tannins mg eq (*)	SD
	Ext 1	Ext 2	Ext 3	Ext 4	Ext 5		
Alnus acuminata	45.7	39.0	11.7	3.5	0.1	235.3	15.4
Clidemia ciliata	60.8	31.7	7.2	0.3	ND	324.0	29.1
Clidemia flexuosa	49.2	34.0	13.5	3.3	ND	266.6	31.2
Clidemia hirta	84.8	14.9	0.2	0.1	ND	521.3	84.3
Miconia tuberculata	46.8	35.7	11.1	3.4	ND	358.2	19.9
Monochaetum meridensis	25.1	48.9	20.0	6.0	ND	311.4	59.9
Psamisia penducifolia	50.6	26.7	19.8	2.9	ND	198.6	34.2
Pteridium arachnoideum	77.5	18.6	2.1	ND	-	20.4	4.4

(*) Equiv of purified quebracho tannin per g of dry leaf.

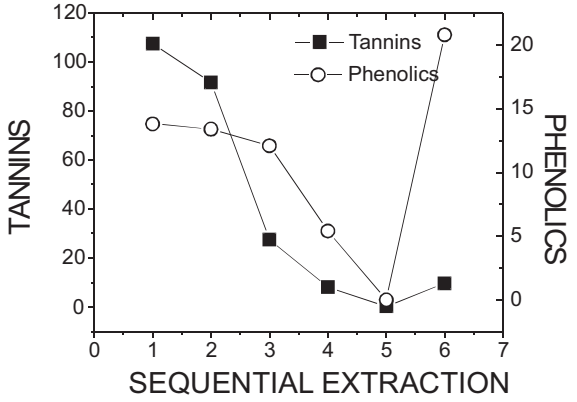

Figure 1. Yield of sequential extractions of *Alnus acuminata* leaves with aqueous acetone 70% and sonication (see text). Tannins: mg –quebracho tannin eq– g^{-1} dw, solid squares. Phenolics: mg –salicylic acid eq– g^{-1} dw.

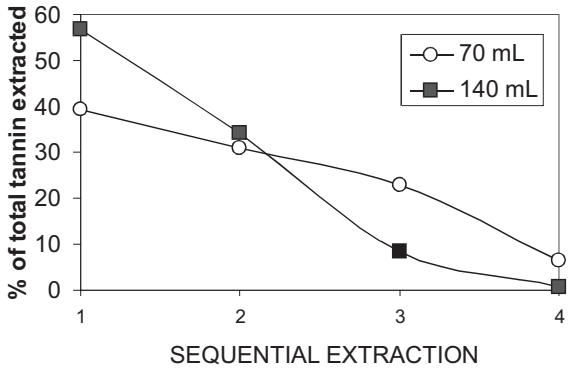

Figure 2. Yield of tannins, expressed as percentage of total recovered, obtained during sequential extractions of *Monochaetum meridensis* leaves (5 g) with 70 and 140 mL of 70% aqueous acetone in 30 min periods per batch.

content of tannin and other phenolics in the dark, tough leaves as it occurs in other trees of this genus (43). The first extraction of its leaves yielded 107.5 mg of quebracho tannin equivalents per g of dry leaf (mg eq g^{-1}) but an additional 91.70 mg eq g^{-1} were recovered in the second extraction. Only by the sixth solvent treatment was a negative Prussian Blue reaction achieved (Table 1). Simple phenolics also proved difficult to pull out from leaf tissue (Figure 1) requiring four consecutive solvent treatments to exhaust their source. However, when a three-hour sonication period was applied to the remaining mull,

Table 3
Variation of the extraction efficiency of *Monochaetum meridensis* leaf phenolics with time of single solvent exposure. Quantities are in mg salicylic acid eq. g^{-1} dw of leaf

Time (h)/Repl	1	2	3	4	5	AVG	SD
0.5	35.5	40.0	38.0	37.5	38.0	37.8	0.93
24	34.4	39.8	35.0	37.5	34.0	36.1	1.05
72	32.5	43.0	39.1	46.5	42.5	42.7	3.15
120	42.5	34.5	42.5	40.9	40.9	39.9	1.65

which was presumably exhausted, an additional 31.8% was added to the total phenolic material recovered. This suggests that a sizable portion of these phenolics were bound to cell components and/or protein and was separated likely by enzymatic or natural hydrolysis. This was the only species where this phenomenon was observed.

The Melastomataceae is a well represented family in mountain areas of the neotropics with over 180 species of the genus *Miconia* in Venezuela alone (44). Most of these plants are successional shrubs and small trees. The leaves of the five common Melastomataceae species selected among those containing significant quantities of phenolics were extracted exhaustively until all of this material was pulled out into solution (Table 1). Phenolics in each species behaved differently in their yield per sequential extract. While *Clidemia ciliata* and *C. hirta* furnished the great majority of their phenolic material in the first treatment, *Miconia tuberculata* gave only moderate amounts in about equal quantity in the first two extractions. Condensed tannins (Table 2) also opposed difficulties to extraction depending on plant species of the studied Melastomataceae. For example, most tannins from *C. hirta* could be extracted from leaf tissue in only two solvent batches but *M. tuberculata* and *C. ciliata* were more demanding to release their tannins content.

Phenolics in *Clidemia flexuosa* and *Monochaetum meridensis*, and also tannins in the latter displayed an unexpected solubility pattern, as the second extraction furnished a significantly *higher* yield of material than the first. *M. meridensis* was further examined to explain this. First, extended contact of the blended leaves with solvent (Table 3) gave no variation in the amount of total phenolics drawn into solution. Secondly, increasing the amount of solvent/sample (v/w) ratio from 70/5 to 140/5 (mL/g) raised the efficiency of the first total phenolic removal from the leaves from 39.5% to 56.7% and reduced the number of successive extractions necessary for complete pull out of material from four to three (Figure 2). However, no improvement in phenolics/tannin yield could be accomplished by increasing this ratio. The odd response of these plants to extraction with aqueous acetone could be due to two possible causes: 1) During blending, plant tissue became disrupted to the point of releasing hydrolytic enzymes into the medium capable of severing covalent bonds between cell-wall carbohydrates or protein and phenolic derivatives (45). As the reaction should be time-dependent, a higher yield of the liberated compound would be attained as the contact time between leaf tissue and solvent progressed. 2) Phenolic/tannin derivatives might not be soluble enough leading to saturation of the first solvent batch that also car-

ries into solution other more soluble, competing solutes not active to the Prussian Blue test. Their interference in the second solvent batch would be reduced by their lower concentration.

If enzymatic cleavage would have been involved, extended exposure of the crushed plant material to the solvents would result in the gradual release of phenolics. However, the results of Table 3 showed that no additional recovery of these compounds could be procured after five days of contact at room temperature, thus making unlikely this proposition. Additionally, the higher yield of condensed tannins obtained after doubling the amount of extraction solvent in the first batch (Figure 2) indicated that solubility of the particular compounds contained therein was indeed the limiting factor. It is to be noted that a proportional rule for increasing solvent/plant tissue ratio and expecting a higher extract yield, could not be applied for practical purposes, because blending and rupture of leaf suspended particles became much less efficient at ratios higher than 140/5 (v/w).

Psamisia penducifolia, also a commonly found forb in the area possessed a larger proportion of reactive leaf phenolics (Table 1) than *A. acuminata* growing in the same habitat. As opposed to the long lived leafs of this tree, herbaceous *P. penducifolia* develops fast growing leaves that last only for the 8-9 rainy months, which according to existing optimal defense theories should not be allowed enough time to accumulate significant quantities of constitutive defenses such as tannin. In fact, the metabolism in this plant's leaves appears adapted to provide an exacerbated phenolics synthesis likely in response to the prevailing conditions of intense UV-B radiation in the 2000-2500 m altitudinal band of the study area (19).

Pteridium arachnoideum, as many other ferns, synthesizes and accumulates increasing amounts of phenolics and tannins in the fronds as they mature, but appears as the least effective of the studied group of species. To metabolic and genetic constraints may also contribute the relatively shorter period of growth and decay of these fronds (3-4 months) to the lower capacity to accumulate these compounds.

Finally, in all studied species, except mature fronds of *Pteridium arachnoideum*, tannins appeared in a larger proportion or reacted more actively in the oxidative Prussian Blue process than monomeric phenolics, and the tannin/phenolics ratio was species dependent. This result suggested that the respective biochemical paths are not controlled to the same extent by biochemical regulation under excess high frequency solar radiation or other environmental demands.

Conclusions

The selected heliophytes of the shrub community here studied all contained high levels of phenolics and aqueous acetone-soluble tannins.

These levels occur likely in response to the environmental stress of excess high frequency solar radiation typical of tropical mid and high elevation areas.

Their reactivity towards the Prussian Blue reaction attests to their antioxidant potential that should be studied further in the context of their possible application.

Extraction of monomeric, olygomeric and polymeric phenolics from plant materials should be carried out in sequence until their exhaustion, and the number of batches will depend on the particular species.

Most species studied, in being hardy, common and potentially cultivable in marginal land could become potential sources of useful phenolic/tannin derived compounds of economic interest.

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