BOTANICAL MUSEUM LEAFLETS
HARVARD UNIVERSITY

CAMBRIDGE, MASSACHUSETTS, OCTOBER 31, 1978 VOL. 26, NO. 8

EFFECTS OF FIELD PRESERVATION ON THE FLAVONOID CONTENT OF *JESSENIA BATAUA*

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As a preface to a chemotaxonomic investigation on the *Jessenia-Oenocarpus* complex (Palmae), it seemed desirable to determine how specimens of plants collected in the field in the wet tropics should be best preserved for future chemical investigation.

Up to 30-40 years ago, most collected plant material was dried by placing it in a conventional press with frequent change of blotters to absorb moisture from the specimens, the entire process taking about a week (1). However, in the wet tropics much of the material was damaged by insects or micro-organisms before drying was complete, rendering the specimen of little value for taxonomic purposes. The artificial drying of such specimens by the use of field stoves or even electric bulbs overcame these problems, but collectors could not easily transport such equipment to remote study areas. Thus, the use of chemical preservatives was introduced to treat plants collected in the field and to prevent them from deterioration by insects or by rotting, until they could be dried at a base facility. Schultes (2) suggested a formalin (40% formaldehyde) and water mixture of approximately 1:5 to avoid the spoilage of most plant materials. Moore (3) recommended using a 1% aqueous solution of hydroxyquinoline sulfate, while Hodge (4) cited the use of an ethanol mixture, noting also that the use of

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Botanical Museum Leaflets (USPS 404-990). Published monthly except during July and August by the Botanical Museum, Harvard University, Cambridge, Massachusetts 02138. Subscription: $25.00 a year, net, postpaid. Orders should be directed to Secretary of Publications at the above address. Second-Class Postage Paid at Boston, Massachusetts.

potable spirits worked quite well if nothing else was available.

Although it is certain that such methods are useful for the adequate preservation of plant specimens for classical taxonomic purposes, it appeared likely to us that specimens preserved in such ways might have suffered a change in their chemistry. For example, formaldehyde is known to react with many aromatic compounds, while treatment with aqueous alcohols (ethanol and methanol) is the method of choice for extracting a wide variety of secondary compounds, used in chemotaxonomic studies, from plant tissues. Consequently, the use of such methods of preservation might make it difficult to properly survey the many different classes of such compounds which have been used for systematic purposes (5). Flavonoids, for example, as well as related phenolics, might be severely affected. These are probably the most useful class of secondary plant constituents for chemotaxonomic purposes because of their widespread distribution, diversity of structure, and relative ease of identification using simple apparatus (6).

Since the flavonoids have previously been shown to be useful in the taxonomy of the Monocotyledonae, including the Palmae (7), they would appear to be a useful group of compounds to throw light on specific problems relating to the taxonomy of the *Jessenia-Oenocarpus* complex. Since most of the material studied grows in the Amazon Valley, however, many miles from any centers of civilization, it was decided to determine whether the use of plant preservatives in the field would affect subsequent analysis of the material for flavonoid compounds. Preservative treatments similar to those that might be used in the field were, therefore, investigated under laboratory conditions, and the results are presented here.

The plants of *Jessenia Bataua* (Mart.) Burret used in this study were grown from a population of seeds collected from a single tree in Tukunaré, near Mitú, Comisaría del Vaupés, Colombia, in July of 1976. The seeds were germinated, planted in soil and grown under controlled conditions in a phytotron for one year, until the plants were 20 cm. high. Pinnae were removed and subjected to the following treatments in order to simulate the procedures that have been used previously in the

field (2,4): 1. sun dried; 2. dried in an herbarium oven at 105°F; 3. soaked in water; 4. soaked in 95% ethanol; 5. soaked in white rum (86 proof, ca. 44% ethanol); 6. soaked in an FAA mixture (ethanol 90%: glacial acetic acid 5%: formalin 5%); 7. soaked in formalin (100% formalin, ca. 40% formaldehyde) and water in a 1:1 mixture; 8. soaked in formalin alone.

The material for treatments 3-8 was stored in the dark for four weeks at room temperature, and the leaves were then removed from the solutions and air dried. The solutions were kept in a refrigerator.

Two samples of dried leaves from each of the eight treatments described above were finely macerated, extracted with boiling 80% methanol, and the solution concentrated and made up to a final volume of 10 ml. The ultra-violet spectrum (220-400 nm) of the methanol extract (suitably diluted) was determined and the amount of phenolic compounds calculated, using caffeic acid (a cinnamic acid) and rutin (quercetin 3-0-rhamnoglucoside, a flavonol) as standards. Identical amounts of the methanol extracts were also examined by 2-dimensional chromatography, using the solvent systems n-butanol-acetic acid-water 6:1:2 (BAW) followed by 5% aqueous acetic acid (HOAc).

The solutions in which the leaves had been soaked were concentrated to a standard volume and subjected to 2-dimensional chromatography by the procedure described above.

The amount of phenolic compounds remaining in the leaf material following these various treatments is shown in Table 1.

TABLE 1

Treatment	phenols* mgm/gm dry wt.
1. Sun dried	12.5
2. Herbarium dried	11.5
3. Water	3.0
4. 95% Ethanol	2.4
5. White rum	2.4
6. FAA	0.9
7. Formalin-water 1:1	1.8
8. Formalin	1.6

*as caffeic acid

Two dimensional chromatography of the sun dried *Jessenia Bataua* leaf material gave the following pattern of major phenolic compounds. (Figure 1)

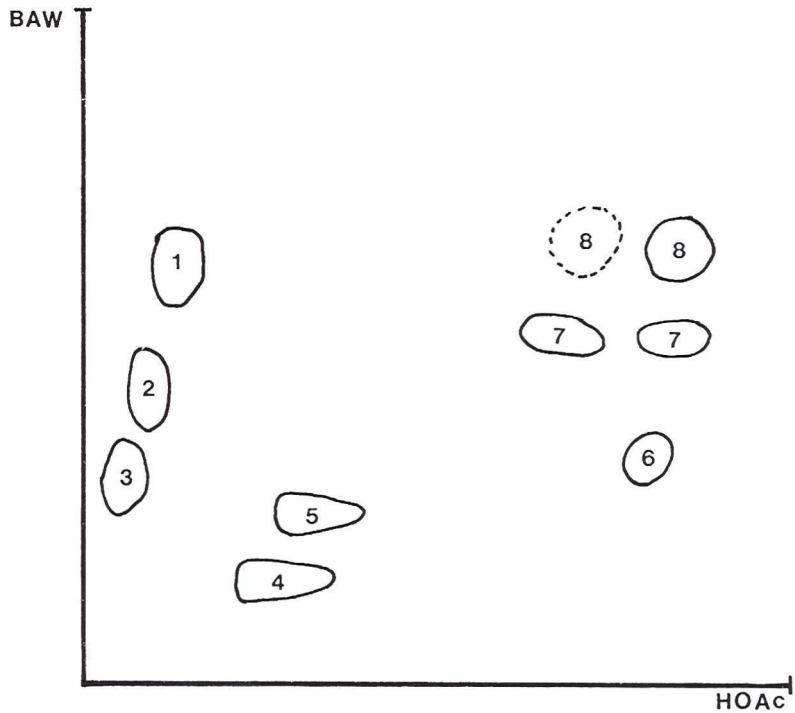

FIGURE 1. 2-dimensional chromatogram of ultra-violet fluorescent spots of flavonoids and related compounds in *Jessenia Bataua*.

From their fluorescence under ultra-violet light and their position on the chromatogram, it is believed that compounds 1-3 are flavonol glycosides; 4-5 flavone glycosides; 6 an unknown cinnamic acid and, compounds 7-8 isomeric forms of caffeic acid and p-coumaric acid derivatives respectively. Further investigations on the identity of these compounds is at present underway.

The 2-dimensional paper chromatograms showed that the following compounds were present in the various leaf extracts (Table 2).

The 2-dimensional paper chromatograms of the solutions in which the leaves had been soaked showed that the following

compounds had been extracted from the leaves and were present in the solution (Table 3).

TABLE 2

Treatment	Phenolic compounds present							
	1	2	3	4	5	6	7	8
1. Sun dried	++	++	++	++	++	++	++	++
2. Herbarium dried	++	++	++	++	++	++	++	++
3. Water	+	+	+	+	+	+	+	+
4. 95% Ethanol	tr	tr	tr	tr	tr	tr	tr	tr
5. White rum	tr	tr	tr	tr	tr	tr	tr	tr
6. FAA	–	–	–	–	–	–	–	–
7. Formalin-water 1:1	–	–	–	–	–	–	–	–
8. Formalin	–	–	–	–	–	–	–	

(++ = very positive; + = positive; tr = trace; – = negative)

TABLE 3

Treatment	Phenolic compounds present							
	1	2	3	4	5	6	7	8
3. Water	+	+	+	+	+	+	+	+
4. 95% Ethanol	++	++	++	++	++	++	++	++
5. White rum	++	++	++	++	++	++	++	++
6. FAA	+	+	+	+	+	+	+	+
7. Formalin-water 1:1	–	–	–	–	–	–	–	–
8. Formalin	–	–	–	–	–	–	–	–

As we expected, there is no doubt from the above results (Tables 1-3) that, if plant material is to be subjected to chemotaxonomic investigation for flavonoids or closely related phenolic compounds, the material must be preserved only by sun drying or by subsequent artificial drying. Soaking in preservatives containing 95% ethanol, white rum and FAA results in changes of cell permeability and consequent leakage of phenolic material into the solution (Table 3). Preservatives containing formaldehyde in high concentration (nos. 7 & 8), which cross link with proteins, carbohydrates and phenolic compounds to give insoluble precipitates, show no free phenolic compounds either in methanolic leaf extracts or in the solutions themselves. Water affects the concentration of the

phenolics in the leaf less, but it is not a good preservative because of its lack of fungicidal or bacteriocidal properties. Therefore, care should be taken when using tropical herbarium material, since, in many cases, the nature of their treatment after collection is unknown.

On the basis of these results, ways of treating of *Jessenia Bataua* for subsequent chemical analysis can be rated as follows: (Table 4; highest rating is 1)

TABLE 4

Treatment	*Rating*
Sun dried Herbarium dried	
Water	2
95% Ethanol White rum	3
Formalin-water 1:1 Formalin	4
FAA	5

Based on our preliminary experiments, the following suggestions can be made:

First: All herbarium material should be plainly marked on the label as to whether it has been chemically treated in the field and if so for how long and in what way. If specimens have been treated they are occasionally so marked, but rarely is it indicated that specimens were merely sun dried or herbarium dried. This lack of information reduces the value of these specimens as potentially useful samples for chemical study.

Second: With the increasing use of chemotaxonomy as one of the tools for systematics, at least 10 gms. and preferably 50 gms. of leaf material (or flowering material,

or both depending on the plant) should, whenever possible, be collected, sun or 'blotter' dried separately, and affixed in an air tight bag to the original specimen for later chemotaxonomic use. This is especially urgent for tropical material, since the habitats listed on herbarium sheets often no longer exist. As flavonoids have been identified in herbarium specimens over 100 years old, there is little problem of subsequent shortage! A few hours of extra field effort in caring for this material might then help towards solving taxonomic problems which could arise in the future, especially during expeditions of "salvage botany", collecting from important localities due to be destroyed by colonization or programs of "development through destruction".

Obviously, further studies are needed on the use of preservatives covering the entire range of tropical material, including monocotyledyons and dicotyledons, ferns and lower plants, as has in part been done by Giannasi for temperate plants (pers. comm.). With chemotaxonomy less an experimental subject and coming of age as a valid systematic tool, our field methods must be readjusted to provide a source of reliable material for the future investigator. Thus, the use of this material will, it is hoped, serve in our attempt to understand the complex and dynamic biological order of the tropics.

ACKNOWLEDGMENTS

For valuable comments and assistance in preparing this paper, we are grateful to Prof. Richard Evans Schultes and Mr. Michael Donoghue of Harvard University, and to Prof. Tony Swain of Boston University. Mr. Kim Laver, a student at Boston University, assisted in the experimental work. Funding from the following sources for field work in South America is gratefully acknowledged: Centro de Desarrollo Integrado "Las Gaviotas", Colombia, Dr. Paolo Lugari Castrillón, Director; Sigma-Xi, the Scientific Research Society of North America; the Atkins Fund of Harvard University.

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PLATE 20

Plate 20. *Jessenia Bataua (Mart.) Burret* in a swampy habitat near Manáus, Brazil. - Photograph by M.J. Balick.