*Principes*, 35(3), 1991, pp. 142–146

# Phenolic Constituents of Coccothrinax (Palmae)

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ABSTRACT

Flavonoids and phenolic acids were extracted from five species of *Coccothrinax*. Nine compounds (caffeic acid, chlorogenic acid, kaempferol, luteolin, luteolin 7-O-glucoside, orientin, quercetin, tricin, and vitexin) were characterized and identified by a combination of one-dimensional chromatography, UV-VIS spectrophotometry, and high pressure liquid chromatography. Chemical variation was limited, but certain species and species groupings could be distinguished by their chemical profiles.

Chemotaxonomy of plants, that is, the comparison of phytochemical data among species of related genera, families, or orders, has developed over the last thirty years as a useful tool in systematics and phylogeny. Because the techniques for isolating and identifying phenolic compounds are relatively simple and inexpensive, they have been adopted by many morphologically oriented taxonomists as a standard source of comparative data (Harborne and Turner 1984). Because palms are large, woody and tropical, typical morphological data are difficult to collect and many taxonomic problems remain. Thus, chemical approaches may aid in our understanding of palms (Williams et al. 1973; Harborne et al. 1974; Williams et al. 1983; Hirai et al. 1984*a*, *b*; Zona and Scogin 1988; Zona 1990). In these previous studies, two or more congeneric species often were distinguished by phenolic profiles.

*Coccothrinax* Sarg. is a taxonomically problematic genus (Uhl and Dransfield 1987), in which estimates of the number of actual species range from about 20 (Moore 1973) to 50 (Muñiz and Borhidi 1982, Borhidi and Muñiz 1985, Uhl and Dransfield 1987). The genus is restricted to the Caribbean region with a center of diversity in Cuba where 60% of the taxa occur. Because *Coccothrinax* is well represented in Fairchild Tropical Garden, we undertook a preliminary survey of foliar phenolic compounds in a limited sample of species to ascertain whether phenolic constituents have potential as systematic characters in the group.

Little is known concerning phenolics in *Coccothrinax*. Williams et al. (1973) chromatographically surveyed 125 species of palms for hydrolyzed and charged flavonoids. They included 32 species of the tribe Corypheae to which *Coccothrinax* belongs, but they analyzed only one sample of one species of *Coccothrinax*, *C. barbadensis* (Lodd. ex Mart.) Becc. (as *C. dussiana* Bailey). The species yielded leucocyanidin and mixtures of unidentified charged flavonoids and flavone C-glycosides. No other work on *Coccothrinax* is known to us.

## Materials and Methods

*Plant Material.* Leaves were collected from garden-grown plants and air dried.

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All plants are accessions in Fairchild Tropical Garden, and all vouchers are deposited in the Garden's herbarium (acronym FTG) as follows: *C. argentata* (Jacq.) Bailey: acc. no. 58823E, *Nauman 1751. C. inaguensis* Read—sample 1: acc. no. 60802F, *Nauman 1749*; sample 2: acc. no. 64284G, *Nauman 1759*; sample 3: acc. no. 5842D, *Nauman 1757. C. jamaicensis* Read: acc. no. 79333A, *Nauman 1747. C. miraguama* (HBK) León—sample 1: acc. no. 70482A, *Nauman 1752*; sample 2: acc. no. 70482B, *Nauman 1758*; sample 3: acc. no. 5861M, *Nauman 1754*; sample 4: acc. no. 58819, *Nauman 1755. C. readii* Quero: acc. no. 591031A, *Nauman 1748.*

The plant samples were chosen to check chemical diversity at several levels of relationship.

*Extraction, Isolation and Identification of Flavonoids.* From each sample, 35 g of dried leaves were ground and extracted with 75% aqueous MeOH (230 ml) under reflux. The extracts were evaporated to dryness under vacuum, then the residue (ca. 5 g) was extracted with hot water. After 24 hours of refrigeration, the precipitate was filtered off, and the clear filtrate was extracted by shaking with ethyl acetate, which was evaporated to dryness under vacuum. Flavonoid compounds from the crude ethyl acetate extracts were separated by one-dimensional chromatography on TLC polyamide sheets with $CHCl_3$-MeOH-$EtCOH_2$-$Ac_2CH_2$ (25:10:5:1 v/v; system 1).

All flavonoid bands were eluted with MeOH. The filtrates were analyzed by co-chromatography and UV spectral analysis. One-dimensional co-chromatography with authentic compounds was performed on Whatman No. 1 paper in three systems, *n*-BuOH-AcOH-$H_2O$ (4:1:5 v/v, BAW, organic phase), *n*-BuOH-AcOH-$H_2O$ (40:100:7 v/v), and 15% AcOH; and on TLC polyamide 6-$UV_{254}$ in two systems, $CHCl_3$-MeOH-$EtCOH_2$-$Ac_2CH_2$ (25:10:5:1 v/v) and $C_6H_6$-$Et_2O$-MeOH-$CHCl_3$ (2:1:1:1 v/v). Standard UV spectral analyses (240–400 nm) using the classical shift reagents (Mabry et al. 1970) were performed on a Perkin-Elmer Lambda 4B UV-VIS spectrophotometer.

Flavone C-glycosides were further characterized by high pressure liquid chromatography (HPLC). Twenty μl of each fraction containing the flavonoids were injected into the HPLC (Hewlett-Packard Model 1090M). Separations were performed on a Hypersil ODS column (5 μ; 4.6 × 100 mm), at 40° C using a water/acetonitrile solvent gradient from 0% to 100% acetonitrile over 10 minutes. Both solvents contained 1% acetic acid. The chromatograms and UV-VIS spectra of individual compounds were recorded with a Hewlett-Packard diode array detector.

The sugar of the flavone 7-O-glycoside was removed by acid hydrolysis and characterized. The compound was refluxed for two hours with 2 N HCl, cooled, and extracted with ether. The concentrated ether layer (dried over sodium sulfate) was co-chromatographed with known markers in one dimension on Whatman No. 1 paper in two systems, 60% AcOH, and AcOH-conc. HCl-$H_2O$ (30:3:10 v/v; Forestal system); and on TLC polyamide sheets in $CHCl_3$-MeOH-$EtCOH_2$-$Ac_2CH_2$ (25:10:5:1 v/v). The aglycone was identified by direct comparison of $R_f$ and color reactions in UV light with and without ammonia. The aqueous layer was neutralized with barium carbonate, filtered and concentrated to about 1 ml, then was co-chromatographed against standards of sugars on silica gel TLC plates in EtOAc-$H_2O$-MeOH-HOAc (13:3:3:4 v/v) and pyridine-EtOAc-$H_2O$ (6:3:2 v/v). The sugar was visualized by spraying with aniline phthalate.

Phenolic acids were separated on TLC polyamide sheets with system 1, eluted with MeOH, and identified by means of UV spectra, color reactions and co-chromatography with standards (Kowalska 1971).

*Table 1. Distribution of phenolic compounds among the samples of* Coccothrinax. Compounds: 1 = orientin, 2 = vitexin, 3 = tricin, 4 = luteolin, 5 = luteolin-7-O-glucoside, 6 = quercetin, 7 = kaempferol, 8 = caffeic acid, 9 = chlorogenic acid. + = present, − = absent, ? = uncertain.

| | Compounds | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Flavone C-glycosides | | Flavones | | | Flavonols | | Phenolic acids | |
| Taxa | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| *C. argentata* | + | + | + | − | − | + | + | − | + |
| *C. jamaicensis* | + | + | + | − | − | + | + | + | + |
| *C. readii* | + | + | + | − | − | + | + | − | + |
| *C. inaguensis* sample 1 | + | + | + | − | + | + | ? | − | + |
| sample 2 | + | + | + | − | + | + | ? | − | + |
| sample 3 | + | + | + | − | + | ? | ? | − | + |
| *C. miraguama* sample 1 | + | + | + | + | − | − | − | + | + |
| sample 2 | + | + | + | + | − | − | − | + | + |
| sample 3 | + | + | + | + | − | − | − | + | + |
| sample 4 | + | + | + | ? | − | − | − | ? | + |

## Results and Discussion

A total of approximately 11 phenolic constituents appeared in preliminary chromatographs. Nine of these were isolated, characterized, and identified (Table 1). These nine represent C-glycosyl flavones (orientin, vitexin), flavones (luteolin, luteolin 7-O-glucoside, tricin), flavonols (quercetin, kaempferol), and phenolic acids (caffeic acid, chlorogenic acid).

Samples 1 and 2 of *C. miraguama* are separate plants from the same seed lot (either from same parent tree or different trees in same population). The identical profiles document consistency of the phenolic constituents among genetically similar plants.

Within *C. miraguama*, the first two samples can be identified with subsp. *havanensis* (León) Borhidi & Muñiz, the third with subsp. *roseocarpa* (León) Muñiz & Borhidi, and the fourth with subsp. *miraguama*. In that sample, the questionable occurrence of luteolin and caffeic acid is due to the heavy concentration of comigrating compounds. If present, these compounds suggest a species-specific uniform profile; if absent, they suggest differentiation among subspecies. Either distributional pattern is consistent with the taxonomy (Nauman and Sanders 1991).

Likewise, the three samples of *C. inaguensis* present a more or less consistent species-specific profile. In sample 3, quercetin was much less concentrated than in the other two samples, and was partly obscured by the other compounds. In all three samples, faint, partly obscured spots were also present in the position of kaempferol. However, if present, kaempferol is not accumulated in as large amounts as in *C. argentata*, *C. jamaicensis* or *C. readii*.

Based on recent morphological comparisons (Nauman and Sanders, 1991, in press), *Coccothrinax* is considered to comprise three major species groups—the argentea-group, represented by *C. barbadensis* in the study by Williams et al. (1973); the pauciramosa-group, represented by *C. miraguama*; and the argentata-group, represented by the remaining four species. Within the argentata-group, *C. jamaicensis* and *C. readii* appear to be very closely related, possibly conspecific. Among the sampled species of the

argentata-group, *C. argentata* is thought to be next most closely related to the pair; and *C. inaguensis*, least closely related.

In the systematic context of the argentata-group, the similar phenolic profiles of *C. jamaicensis* and *C. readii* support their close relationship. They differ only by the occurrence of caffeic acid in the former. These two species share with *C. argentata* the accumulation of the flavonol aglycones quercetin and kaempferol. *C. inaguensis* is unique in possessing luteolin 7-O-glucoside, but it is similar to the other three in the occurrence of quercetin and the probable occurrence of kaempferol. Thus, the data support a closer relationship among the first three than between any of these to *C. inaguensis*.

*Coccothrinax miraguama* is distinctive in having caffeic acid and luteolin as an aglycone; whereas, *C. barbadensis* is distinguished by the loss of tricin. Whether these differences characterize only these individual species or all of the species in their respective species groups is unknown.

Because orientin, vitexin, tricin, and chlorogenic acid (and possibly caffeic acid) occur in at least two of the three species groups, these compounds appear to compose a basic profile for the genus. Individual species either have the basic profile unchanged or have it modified by the addition or loss of one or two compounds. The total number of additional compounds identified in our sample is only five. Thus the diversity of profiles may be rather limited in the genus. The report by Williams et al. (1973) of unspecified C-glycosyl flavones in *C. barbadensis* is consistent with our recognition of orientin and vitexin.

However, Williams et al. (1973) also document that C-glycosyl flavones pervade the whole family. Thus, this class of flavonoids serves not to characterize *Coccothrinax* but indicates its evolution early in the history of palms, and it may indicate a common ancestory of the Palmae and Poales. Likewise, because tricin is quite common throughout the Palmae (although tending to be absent from the Arecoideae), it, too, is not a marker for *Coccothrinax*.

Our techniques and those of Williams et al. (1973) do not allow the comparisons of negatively charged flavonoids, leucoanthocyanins, and phenolic acids. Also, because we examined only small leaf samples (ca. 35 g dry wt.), the compounds listed are those that accumulate in leaf tissues; trace compounds are not reported.

The total profile of only nine to eleven compounds lacks sufficient complexity to distinguish all 50+ taxa in the genus. However, the patterns established in this study suggest that, while only certain species can be distinguished by one or possibly two distinctive compounds, proposed relationships can be supported by the systematic distribution of phenolics. Therefore, we conclude that a broader taxonomic sampling concomitant with a complete characterization of phenolic constituents can assist in solving some systematic problems in *Coccothrinax*.

## Acknowledgments

The South Florida Chapter of the International Palm Society generously supported this work with two grants. Dr. Kelsy Downum provided his HPLC and spectrophotometric facilities, and he read an earlier draft of the manuscript.

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