



Cuticular *n*-alkane in leaves of seven Neotropical species of the family Lecythidaceae: a contribution to chemotaxonomy

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ABSTRACT

Biosynthesized from very long-chain fatty acid wax precursors, *n*-alkanes make a valuable contribution to the taxonomy of plants. The alkane components of foliar epicuticles of seven Neotropical species of Lecythidaceae were investigated: *Bertholletia excelsa*, *Cariniana legalis*, *Couroupita guianensis*, *Eschweilera alvimii*, *Eschweilera ovata*, *Gustavia augusta* and *Lecythis pisonis*. Specimens were collected in the metropolitan area of Recife, Pernambuco, and their *n*-alkane fractions were analyzed by gas chromatography. The chemical relationships among the species were then evaluated using cophenetic correlation and UPGMA. Among the seven species, a total of 15 *n*-alkanes, with 21-35 carbon atoms, were identified and formed a consistent group of *B. excelsa*, *C. guianensis*, *E. ovata*, *G. augusta*, and *L. pisonis* with *n*-C31. The greatest similarities were found between *B. excelsa* and *L. pisonis*, and between *C. guianensis* and *G. augusta*. Nevertheless, a phenetic analysis based on a larger number of species is needed to better understand the chemotaxonomic value of epicuticular *n*-alkanes within the Lecythidaceae.

Keywords: Lecythidaceae, Neotropics, taxonomy, tropical rain forest, wax

The family Lecythidaceae is pantropically distributed, with 10 genera and 118 species, most of them native to Brazil, with the highest diversity in the Neotropical region (Smith *et al.* 2010). In the state of Pernambuco (Northeast Brazil), six genera and 14 species are found (Barbosa 2006), among which *Gustavia augusta* L., *Eschweilera ovata* (Cambess.) Miers, and *Lecythis pisonis* Camb. are highly prevalent in Atlantic Forest areas (Rocha *et al.* 2008;

Silva & Rodal 2008).

Previous phytochemical studies of species of the Lecythidaceae family have reported the presence of alkaloids, terpenoids (volatile oils, diterpenes, pentacyclic triterpenoids and steroids), proanthocyanidins, flavonoids, and other phenolic substances (Costa & Carvalho 2002; Janovik *et al.* 2011; Ferreira *et al.* 2014).

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Among these components, alkanes from epicuticular waxes have acquired wide acceptance as indicators of taxonomic relations between different plant groups (families, genera and species) (Maffei 1996a; Medina *et al.* 2006; Li *et al.* 2012). *n*-alkanes are biosynthesized from very long-chain fatty acid (C > 22:0) wax precursors by the decarbonylation pathway (Kunst & Samuels 2003). However, inconsistencies of using alkanes of plant waxes as taxonomic markers have been pointed out. Some authors have observed that the alkane distribution can be strongly affected by several factors, among them the age of the plant organ (Stocker & Wanner 1975; Nordby & Nagy 1977; Jenks *et al.* 2001). This molecular alteration in the amount and distribution of alkanes in plant leaves can complicate the application of *n*-alkanes as taxonomic markers (Li *et al.* 2013).

To our knowledge, no chemical studies concerning the epicuticular *n*-alkane profile have been carried out with species of Lecythidaceae.

In this study, the composition of foliar epicuticular alkanes of seven Neotropical Lecythidaceae species were studied: *Bertholletia excelsa* Bonpl. (PEUFR 50869), *Cariniana legalis* (Mart.) Kuntze (PEUFR 50870), *Couroupita guianensis* Aubl. (PEUFR 50625) *Eschweilera alvimii* Mori (PEUFR 50624), *Eschweilera ovata* (Cambess.) Miers (PEUFR 50498), *Gustavia augusta* L. (PEUFR 50499) and *Lecythis pisonis* Camb. (PEUFR 50633). The specimens were collected in the metropolitan area of Recife (7°94'37"S, 34°88'17"W) in the state of Pernambuco, Brazil, between March 2010 and June 2012. Exsiccates of these species were deposited at the Vasconcelos Sobrinho Herbarium (PEUFR) of the Biology Department of Federal Rural University of Pernambuco (UFRPE).

To obtain the cuticular wax, individual totally expanded whole fresh leaves from each species, with five replicates, underwent two successive washings during 30 s with 800 mL of dichloromethane (CH₂Cl₂) (Souza *et al.* 2010). *n*-alkane fractions were separated by TLC and analyzed by GC/EIMS (Shimadzu 17A, Kyoto, Japan). The alkane peaks were identified by comparison with authentic samples of *n*-alkane standard solution C21-C40 (Fluka S.A, Costa Rica) and mass spectrometry (NIST05, Standard Reference Database). The analyses were performed with a DB-Wax fused silica capillary column (polyethylene glycol, 30 m × 0.25 mm,

5 % phenyl-95 % dimethylpolysiloxane) with helium at a flow rate of 1 cm³.min⁻¹ and split ratio 1:100. Injector and detector temperatures were 300 °C. The temperature of the column moved from 100 °C (3 min) to 230 °C at 3 °C.min⁻¹ and was maintained at the final temperature under isothermal conditions for 20 min.

The *n*-alkane distribution was analyzed through the unweighted pair group method with arithmetic mean (UPGMA) and Euclidean taxonomical distances. The cophenetic correlation was generated (COPH algorithm) to verify the goodness of fit between the groups in the dendrogram and the similarity matrix coefficient. All analyses were carried out using the software NTSYS version 2.11X (Rohlf 2005).

A total of 15 *n*-alkanes with 21-35 carbon atoms were identified. Long-chain *n*-alkanes prevailed, particularly hentriacontane (*n*-C31). In two species (*C. guianensis* and *C. legalis*), tritriacontane (*n*-C33) was the most prevalent, and in *E. alvimii*, heneicosane (*n*-C21) represented over 60 % of the cuticular alkanes identified. The quantitative distribution of the *n*-alkanes from the seven Lecythidaceae species studied is shown in Table 1.

A high cophenetic correlation coefficient (rcoph = 0.964) was found, which suggests a good fit between the data matrix (Tab. 1) and the phenogram obtained (Fig. 1). The *n*-alkane profile of *E. alvimii* enabled isolating it from a large group formed by most species. The main reason for this isolation was the exceptionally high content of *n*-C21 in *E. alvimii* (over 60 %). Although this species had *n*-C31, the characteristic cuticular alkane of the Lecythidaceae species studied, its content (26 %) was lower than those of five species studied, including *E. ovata*, a species of the same genus, but with high *n*-C31 content (57 %). In contrast, the *n*-C21 content in *E. ovata* was very low. Thus, the *n*-alkane profiles of these two species are very different. It is also worth pointing out the negligible amount of *n*-C31 in *C. legalis*. Although this species was grouped with most of the species studied, *C. legalis* is clearly isolated within this group. Despite the very small *n*-C21 content, the moderate contents of pentacosane (*n*-C25) and heptacosane (*n*-C27) make the alkane profile of *C. legalis* quite peculiar.

The group formed by *B. excelsa*, *C. guianensis*, *E. ovata*, *G. augusta* and *L. pisonis* was characterized by the highest concentrations of *n*-C31 found (38.3 to 57.2 %). In this

Table 1. Distribution and abundance of foliar epicuticular *n*-alkanes in seven Lecythidaceae species (Pernambuco, Brazil).

Species	<i>n</i> -alkanes (%)														
	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34	C35
<i>B. excelsa</i>	0.27	0.33	0.28	0.86	0.91	0.91	1.09	0.80	23.16	3.55	45.55	5.04	16.67	0.23	0.26
<i>C. legalis</i>	0.25	0.65	0.02	3.20	21.65	3.3	19.19	1.49	12.45	2.89	0.22	5.89	28.80	-	-
<i>C. guianensis</i>	-	0.03	0.11	0.27	1.22	0.05	1.82	0.61	6.31	1.48	38.33	4.71	44.13	0.36	-
<i>E. alvimii</i>	62.70	0.06	0.06	0.05	0.17	0.16	0.20	0.23	4.09	1.47	26.09	1.64	3.01	-	-
<i>E. ovata</i>	0.10	0.11	0.10	0.19	0.28	0.17	0.69	0.64	23.71	6.04	57.25	4.73	5.71	0.13	-
<i>G. augusta</i>	0.12	0.31	0.33	0.51	0.62	0.43	0.55	0.50	1.90	0.70	49.69	2.88	41.04	0.14	0.20
<i>L. pisonis</i>	-	0.10	0.14	0.42	0.27	0.25	0.66	0.61	15.14	2.20	49.85	4.35	24.48	0.49	0.91



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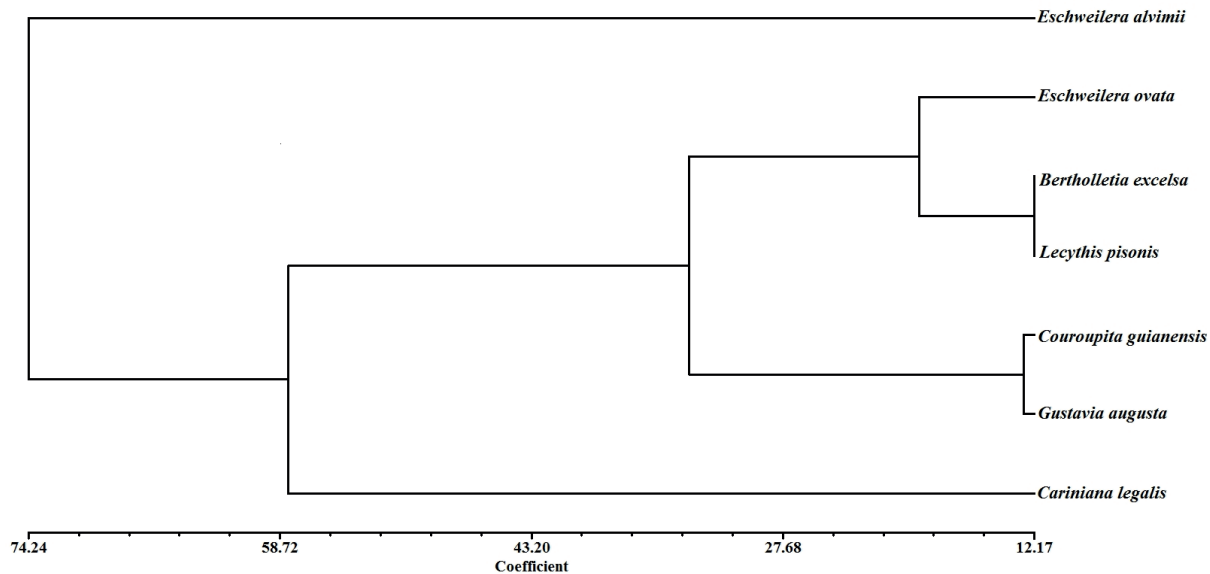


Figure 1. Phenogram of UPGMA clustering of Euclidian distance based on the quantitative distribution of cuticular *n*-alkanes from Lecythidaceae species found in the Atlantic Forest (Pernambuco, Brazil). Cophenetic correlation coefficient ($r = 0.964$).

group, the greater similarities between *B. excelsa* and *L. pisonis* and between *C. guianensis* and *G. augusta* were mainly due to the even distribution of *n*-C31 and *n*-C33.

All species studied here belong to the subfamily Lecythidoideae, which is characterized by genera with fibrous bark, simple, alternate leaves, actinomorphic or zygomorphic flowers, numerous stamens, inferior to superous ovaries, and bitegumented ovules, among other characteristics (Prance & Mori 1979). According to Huang *et al.* (2011), the relations within Lecythidaceae are not fully understood, particularly within Lecythidoideae.

Our findings, for instance, show strong similarity between *C. guianensis* and *G. augusta*. However, *C. guianensis* has zygomorphic flowers, ovules inserted along a bilamellar placenta, and indehiscent fruits with lenticular seeds, while *G. augusta* has actinomorphic flowers and is the only Lecythidaceae genus with poricidal anthers and plano-convex cotyledons. These characteristics, except for the indehiscent fruits in *Couroupita*, are synapomorphies in both genera (Mori *et al.* 2007).

The results also showed high similarity between *B. excelsa* and *L. pisonis*. Cladistic analyses based on anatomic and morphologic data of vegetative and reproductive organs place *B. excelsa* in the same clade as other *Lecythis* species (section *Lecythis* A), but *L. pisonis*, the species analyzed here, and other species of this genus do not belong to this section. *Lecythis* is not monophyletic according to Huang *et al.* (2011).

According to Mori *et al.* (2015) and Huang *et al.* (2015), *E. alvimii* and *E. ovata* are placed in different clades in the Lecythidaceae family (Tetrapetala and Parvifolia, respectively). These species can be differentiated by the

ligule morphology, which is double coiled in *E. ovata* and simple in *E. alvimii*. The distribution of these two species in different sections matches the *n*-alkane profile found (Fig. 1).

Several authors have used *n*-alkane distribution with a chemotaxonomic indicator, such as Maffei (1996b) with species of Apiaceae, Brassicaceae and Leguminosae (Subfam. Papilionoideae), Costa Filho *et al.* (2012) with *Croton* L. (Euphorbiaceae) and Silva *et al.* (2012) with species of the genus *Solanum* Subgen. *Leptostemonum* Dunal (Bitter).

Nevertheless, a phenetic analysis based on a larger number of species is needed to better understand the chemotaxonomic value of cuticular *n*-alkanes in Lecythidaceae.

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