Comparative Study of Lipids in Mature Seeds of Six *Cordia* Species (Family Boraginaceae) Collected in Different Regions of Brazil

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ABSTRACT: The oil content, FA, and lipid class composition of the mature seeds of six *Cordia* species were analyzed. Mature seeds of each species were collected in their natural habitat from 2002 to 2004. The total lipid content varied from 1.9% to 13.2%, there being significant differences between the results found in different years for each species and between the species analyzed. The contents of FFA varied from 2.0% to 7.9% of total lipids. Neutral lipids (NL) were the largest class, making up between 89.6% and 96.4% of the total lipids; the phospholipids (PL) were the second largest class (3.0% to 8.9% of the total lipids), and the glycolipids (GL) were the smallest class (0.6 to 3.4%). The presence of GLA was determined in each class of lipids; it is predominant in the NL. Levels of GLA ranged from 1.2% to 6.8% of total seed FA. This is, to our knowledge the first study of lipid composition in seeds of species of *Cordia* from Brazil.

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GLA, a FA belonging to the omega-6 (ω-6) family, has received considerable attention in recent years due to its beneficial effects on the treatment and control of: cardiovascular disease (1), diabetes (2), atopic dermatitis (3), alcoholism (4), premenstrual syndrome (5), hypertension (6,7), and tumors (8). Studies have also shown that it reduces VLDL, LDL, and cholesterol levels (9,10).

Heath products containing GLA mainly consist of lipid extracts from *Oenothera biennis* L., *Borago officinalis* L. and *Ribes nigrum* L. (11). But other species belonging to the Boraginaceae, Aceraceae, and Ranunculaceae families can also be potential sources of GLA. A study of 36 species, belonging to 20 genera of the Boraginaceae family showed that all the accessions analyzed contained GLA and that GLA was a potential chemotaxonomic marker for this family (12). Although no species of *Cordia* (Boraginaceae) were included in that study, the results suggested that other Boraginaceae species might also contain GLA. Although previous studies reported the FA composition of the seed oil of several wild and cultivated *Cordia* species (13–18), they did not included Brazilian *Cordia* species. In a study of three Brazilian *Cordia* species (*C. ecalyculata, C. myxa,* and *C. selowiana)*, the analysis of the FA composition of seed oil revealed the presence of GLA (11). For this reason, we found it interesting to further evaluate Brazilian *Cordia species* for GLA content.

The objective of this study was to analyze the composition of lipids in mature seeds of six different native Brazilian species of *Cordia,* with emphasis on determining the presence of GLA, and to see whether this acid is found in the reserve lipids.

EXPERIMENTAL PROCEDURES

Sample collection and preparation. Seeds from mature plants of *Cordia* species were collected in different regions of Brazil (Table 1). The species used were *Cordia ecalyculata, C. glabrata, C. sellowiana, C. superba, C. trichotoma,* and *C. goeldiana.* The seeds were peeled, and only the nut and mesocarp were ground and homogenized in a food processor. The obtained powder was immediately analyzed. Three samples were taken from each plant at each location and time of collection, and these replicates were processed individually.

Extraction and quantification of total lipids. Twenty grams of the homogenized powder obtained from the procedure just described were extracted according to the method of Bligh and Dyer (19). Samples were first extracted with 50 mL of chloroform/methanol/water (1:2:0.8, by vol), and then with 50 mL of the same solvent system but with a different proportion (2:2:1.8). The extract was filtered and dried using sodium anhydride, and 10 mL of this extract was transferred to a previously weighed beaker and kept in an oven at a temperature of 105°C until constant weight. Total oil content was measured gravimetrically and expressed as a percentage of dry seed weight.

Separation of different classes of lipids by column chromatography. Lipids were separated following the procedure described by Christie (20). Total lipids were partitioned into classes by column chromatography. A glass column (20 cm long by 1.25 cm i.d.) containing 20 g of silica gel 60 (70–230 mesh, Merck) was used as stationary phase, and chloroform, acetone, and methanol were sequentially applied to the column

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TABLE 1
Place and Date of Collection, Total Lipid Content, and Fractions (g/kg) of the *Cordia* L. Seeds Analyzed[a]

Species	Place of collection[b]	Date of collection	Total lipid content (%)	FFA content (%)	Major lipid classes (% of total)		
					NL	GL	PL
C. *ecalyculata* Vell.	(1)	April 2002	9.7 ± 0.4[a]	2.8 ± 0.2[a,c]	95.0 ± 1.8	1.0 ± 0.6	4.0 ± 1.2
		April 2003	7.3 ± 0.4[b]	3.2 ± 0.3[a,g]	94.1 ± 1.5	0.9 ± 0.4	5.0 ± 0.8
		April 2004	8.4 ± 0.5[c]	3.3 ± 0.2[a,g]	94.7 ± 1.3	1.1 ± 0.8	4.2 ± 1.0
C. *glabrata* DC.	(2)	October 2002	2.6 ± 0.4[d]	7.3 ± 0.2[b,f]	92.3 ± 1.1	1.9 ± 0.8	5.8 ± 1.1
		October 2003	1.9 ± 0.8[d]	7.7 ± 0.4[b]	91.0 ± 1.6	3.2 ± 0.7	5.8 ± 1.2
		October 2004	2.1 ± 0.1[d]	7.4 ± 0.8[b]	91.1 ± 1.8	3.4 ± 1.0	5.5 ± 0.8
C. *goeldiana* Huber	(3)	December 2002	2.2 ± 0.1[d]	7.9 ± 0.7[b]	89.8 ± 1.4	2.8 ± 0.8	7.4 ± 0.8
		November 2003	2.5 ± 0.3[d]	7.6 ± 0.3[b]	90.7 ± 1.7	3.1 ± 1.0	6.2 ± 0.9
		December 2004	2.3 ± 0.2[d]	7.8 ± 0.2[b]	89.6 ± 1.6	3.4 ± 0.5	7.0 ± 0.9
C. *sellowiana* Cham.	(4)	October 2002	13.2 ± 0.7[e]	2.0 ± 0.2[c]	90.6 ± 1.8	2.5 ± 0.8	6.9 ± 1.2
		September 2003	9.5 ± 0.3[a,c]	2.3 ± 0.2[a,c]	91.0 ± 1.7	1.9 ± 0.6	7.1 ± 0.9
		October 2004	9.3 ± 0.3[a,c]	2.5 ± 0.6[a,c]	91.8 ± 1.9	2.6 ± 0.6	5.6 ± 1.0
C. *superba* Cham.	(5)	September 2002	6.0 ± 0.4f	4.6 ± 0.4[d,h,i]	89.7 ± 1.5	3.2 ± 0.9	7.1 ± 1.0
		September 2003	5.3 ± 0.5[f,g]	4.5 ± 0.6[d,i]	89.5 ± 1.7	2.9 ± 1.0	7.6 ± 0.9
		October 2004	4.4 ± 0.4[g,h]	4.1 ± 0.5[d,g]	89.7 ± 1.5	1.4 ± 0.6	8.9 ± 1.2
C. *trichotoma* Vell.	(4)	June 2002	4.3 ± 0.3[g,h]	5.4 ± 0.4[i,e]	96.4 ± 1.7	0.6 ± 0.5	3.0 ± 0.8
		June 2003	3.1 ± 0.2[d,i]	5.6 ± 0.2[e,h]	94.2 ± 1.9	1.8 ± 0.9	4.0 ± 1.0
		June 2004	3.9 ± 0.3[h,i]	6.3 ± 0.6[e,f]	94.6 ± 0.9	2.3 ± 1.0	3.1 ± 0.8

[a]Results are expressed as means ± SD of three measurements. Means within a column that do not share a superscript letter are significantly different ($P \leq 0.05$).
[b](1) São Paulo state (city of Rio Claro); (2) Mato Grosso state (Xingu Indian Reservation); (3) Pará state (city of Boa Vista); (4) São Paulo state (city of Vargem); (5) Minas Gerais state (city of Bueno Brandão).

to elute neutral lipids (NL), glycolipids (GL), and phospholipids (PL), respectively. The lipid fractions were reduced in volume at 40°C using a rotary evaporator. Then 100–300 mg of each fraction were transferred to preweighed flasks; the residual solvent was evaporated under nitrogen, and the samples were placed in a desiccator until constant weight. The content of each lipid class was calculated by weight, and expressed as a percentage of the total lipid dry weight.

FFA content. The FFA content of the lipid fractions was determined colorimetrically according to Kwon and Rhee (21). The total content of FFA in the samples was expressed as oleic acid equivalents using a standard curve.

GLC of FAME. Total lipids were converted into methyl esters of FA using BF_3 methanol as esterifying agent, according to the method suggested by the American Oil Chemist's Society (22). The methyl esters were diluted in hexane and analyzed by GC using a Chrompak chromatograph (model CP 9001) with a flame ionization detector and a CP-Sil 88 capillary column (Chrompak, WCOT Fused Silica 59 m × 0.25 mm). Detector temperature was 270°C, and temperature of the injector was 250°C. Initial temperature was 80°C for 7 min, programmed to increase 10°C per min up to 180°C, hold for 3 min, and then increase 3°C/min up to a maximum temperature of 210°C. Carrier gas used was hydrogen at a flow rate of 2 mL/min. The identification of the FA was done by comparison of the retention times of the sample components with authentic standards of FAME injected under the same conditions. Coelution (spiking) of samples was also used. FA composition, as percentage of total FA weight, was calculated using area counts of the chromatogram.

GC–MS. The FA composition was confirmed by using a Hewlett Packard 5890 GC–MS. FAME were separated using an Ultra-2 (Hewlett Packard) apolar column (25 m length × 0.22 mm internal diameter × 0.33 μm film thickness). The following temperature program used: 1 min at 60°C, then a linear increase from 60°C to 300°C at 10°C/min, and held at 300°C for 5 min. Helium was used as carrier gas. The mass spectrometer used electron ionization (EI) with filament potential of 70 eV and temperature of 100°C, and a quadrupole mass analyzer in rf/DC mode. Components of the sample were identified by comparison with data from mass spectrometry libraries.

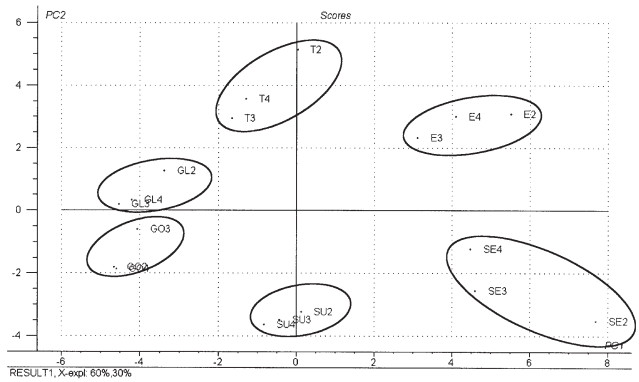

FIG. 1. PCA of the data in Table 1. Data for three samples of each species are shown. T2, T3, and T4: *C. trichotoma* Vell.; E2, E3, and E4: *C. ecalyculata* Vell.; SE2, SE3, and SE4: *C. sellowiana* Cham.; SU2, SU3, and SU4: *C. superba* Cham.; GO2, GO3, and GO4: *C. goeldiana* Huber; GL2, GL3, and GL4: *C. glabrata* DC. Variables analyzed: place of collection, total lipid content (%), FFA content (%), and major lipid classes (% of total).

Statistical analysis. One-way ANOVA and Tukey's studentized range test were used to determine the differences in mean values for each sample based on data collected from three replications of each sample. Significance was established at $P \leq 0.05$.

Principal component analysis (PCA) of the data in Table 1 was performed using the 2.60 version of Pirouette software from Infometrix (Woodinville, WA), in order to group the samples. The data was preprocessed using autoscale and the PCA method was run.

RESULTS AND DISCUSSION

The total lipid contents, FA contents, and contents of the main classes of lipids detected in the mature seeds of six *Cordia* species are shown in Table 1. The total lipid content varied between 1.9% and 13.2%, there being significant differences between the results found in different years for each species and between the species analyzed. Based on these results, the samples can be divided into three groups: those with total lipid content of up to 2.5% total seed weight (*C. glabrata* and *C. goeldiana*); those with total lipid content between 3% and 6% (*C. superba* and *C. trichotoma*), and those with total lipid contents over 7% (*C. ecalyculata* and *C. sellowiana*).

Of these, *C. ecalyculata* and *C. sellowiana* seeds collected in 2002 had significantly higher total lipid content than the seeds collected in the following years (2003 and 2004). For *C. superba, C. glabrata,* and *C. trichotoma,* the same trend was observed, but not for *C. goeldiana.* The differences, however, were not statistically significant in relation to both of the following years. It is known that different climatic conditions, such as rainfall, temperature, soil, and stage of germination of the seeds, can cause significant variations in the lipid contents and composition of seeds of the *Boraginaceae* family (23,24,25). According to meteorological bulletins reported by the governmental agency INPE (National Space Research Institute of the Brazilian Science and Technology Ministry), the year 2002 was the hottest since 1860, and rains were below average in most of Brazil the same year. On the other hand, the years 2003 and 2004 presented higher rainfall than usual for the state of São Paulo (26). Apparently a hotter and drier climate resulted in higher lipid content, although other factors may also be involved in this result.

PCA of the data in Table 1 was run using the following as variables: the place of collection, total lipid content (%), FFA content (%), and the percentages of each lipid class (PL, GL, and NL) for each sample analyzed. The samples were grouped according to their species, indicating that the differences between species were greater than variations between years within each species due to differences in meteorological conditions. PC1 explains 60% of the variability, and PC2 explains 30%.

In relation to total lipid content, the results obtained for *C. superba* (4.4–6.0%) and *C. trichotoma* (3.1–4.3%) seeds are similar to those found by Kleinman *et al.* (16) for seeds of *C. obliqua* Wild (4.0%) and by Mukarram *et al*. (17) for *C. myxa* (3.0%) seeds. Other authors reported higher lipid contents: Daulatabalad *et al.* (15) found 18.0% for *C. rothii.* Mayworm *et al*. (18) reported 19.6% lipid content for seeds of *C. glabrata* A. DC. from the semi-arid region of the Brazilian northeast. A recent study (11) reported 25.8% lipid content in fresh *C. sellowiana* seeds collected in the same region at the same time of the year as Mayworm *et al.* (18). Some plants of the Boraginaceae family are known for their high lipid contents, especially *Myosotis discolor* Pers. (34.1%) and *M. sylvatico* Hoffm. (31.8%) (12). Guil-Guerrero *et al.* (27) analyzed 20 species collected in Spain belonging to the Boraginaceae family and reported lipid contents of 6.6% for *Echium humille* and 30.7% for *Borago officinalis*, but no samples of *Cordia* were analyzed.

The contents of FFA varied between 2.0% and 7.9% of total lipids. NL were the largest class, making up between 89.6–96.4% of the total lipids, the PL were the second largest class with 3.0–8.9% of the total lipids, and the GL the smallest class (0.6– 3.4%). The presence of GLA vas determined in each class of lipids; it is predominant in the NL, making up 95–98% of the FA content of this class of lipids (data not shown). To our knowledge no previous studies have reported the classes of lipids found in seeds of *Cordia.* These results are comparable to those obtained by Senanayake and Shahidi (25) for *Borago officinalis* L. seeds, another member of the same family (Boraginaceae), with an average of 84.1% of NL, 8.6% of PL, and 7.3% of GL in relation to total lipids. The results of Hamrouni *et al.* (24) differ, as they did not report the presence of glycolipids for *Borago officinalis* L seeds.

Table 2 presents the relative composition of the FA found in the six species of *Cordia*. In all species, oleic acid is the predominant FA, ranging from 37% to 48%, followed by linoleic acid (17–25%) and palmitic acid (15–20%). Alpha-linolenic (18:3n-3) and stearidonic (18:4n-3) acids were present in all analyzed *Cordia* species. GLA contents ranged from 1.2% in *C. sellowiana* to 6.8% in *C. ecalyculata*, and was not found in *C. trichotoma*. The variations in GLA content between seeds of the same species collected in different years were not significant at $P = 0.05$.

The predominance of oleic acid was also reported by Mayworm *et al.* (18) for seeds of *C. glabrata* A. DC. (40.8% of total FA), a species from the northeast of Brazil. Other authors have reported very different FA compositions in other species of *Cordia*. In the seed oil of *C. rothii*, linoleic acid was the main FA (40%), followed by palmitic (32.8%), ricinoleic acid (10.8%), and oleic acid (7.8%) (15). Alpha-linolenic acid (24.1%), eicosenoic acid (17.5%), and arachidic acid (8.7%) were found to be predominant in the FA composition of *C. myxa* seeds by Mukarram *et al.* (17), who did not report the presence of GLA in these seeds. Presence of GLA was also not reported in seeds of *C. rothii* (syn. *C.angustifolia)* (15) collected in India, nor in *C. salicifolia* (syn. *C. ecalyculata*) and *C. verbenacea* collected in Beltsville, Maryland (16), nor in seeds of *C.glabrata* A. DC. from the northeast of Brazil (18). Only low concentrations of GLA were found for seeds of *C. obliqua* Wild (0.1%) collected in Beltsville, Maryland, by Kleinman *et al.* (16). However, in a previous study carried out

TABLE 2
FA Composition of the *Cordia* L. Seeds Analyzed

Species	Collection date	FA composition (% of total FA)											
		14:0	16:0	16:1n-7	18:0	18:1n-9	18:2 n-6	18:3n-3	18:3n-6	18:4n-3	SFA	MUFA	PUFA
C. *ecalyculata* Vell.	April 2002	2.1 ± 0.6	18.3 ± 0.2	1.0± 0.6	5.2 ± 0.6	45.1 ± 1.0	17.2 ± 0.9	3.6 ± 0.2	6.2 ± 0.5	1.3 ± 0.3	25.6	46.1	28.3
	April 2003	1.9 ± 0.5	15.0 ± 0.7	1.3± 0.3	4.9 ± 0.5	46.8 ± 0.5	18.2 ± 0.8	4.0± 0.3	5.7± 0.4	2.2 ± 0.5	21.8	48.1	30.1
	April 2004	1.5 ± 0.5	14.6 ± 0.7	1.1 ± 0.2	5.0 ± 0.9	47.6 ± 0.9	19.7 ± 0.9	2.3 ± 0.5	6.8 ± 0.7	1.4 ± 0.2	21.1	48.7	30.2
C. *glabrata* DC.	October 2002	1.8 ± 0.5	14.9 ± 0.5	1.8 ± 0.4	7.5 ± 0.8	42.9 ± 0.6	21.6 ± 1.2	3.0 ± 0.6	3.8 ± 0.4	2.7 ± 0.3	24.2	44.7	31.1
	October 2003	1.2 ± 0.4	19.8 ± 0.5	1.5± 0.3	6.9 ± 1.5	37.1 ± 1.5	24.0 ± 0.9	2.5 ± 0.5	4.8 ± 0.5	2.2± 0.3	27.9	38.6	33.5
	October 2004	1.1 ± 0.5	17.4 ± 0.5	1.0 ± 0.5	6.2 ± 1.3	42.2 ± 0.7	23.4± 0.7	2.3± 0.4	3.9 ± 0.5	2.5 ± 0.4	24.7	43.2	32.1
C. *goeldiana* Huber	December 2002	1.6 ± 0.5	14.6 ± 0.5	1.9 ± 0.4	8.1 ± 0.4	40.4± 0.6	23.0± 1.3	2.3± 0.3	5.2 ± 0.4	2.9 ± 0.5	24.3	42.3	33.4
	November 2003	2.0 ± 0.4	16.9 ± 0.5	1.5 ± 0.4	4.0 ± 0.5	44.8± 1.2	20.3 ± 1.2	3.2 ± 0.5	5.8 ± 0.3	1.5 ± 0.4	22.9	46.3	30.8
	December 2004	2.1 ± 0.6	15.8 ± 0.6	1.7± 0.5	6.3 ± 0.7	41.2± 0.7	22.4 ± 0.3	2.5 ± 0.5	5.5 ± 0.8	2.5 ± 0.5	24.2	42.9	32.9
C. *sellowiana* Cham.	October 2002	1.8 ± 0.5	18.0± 0.8	2.3± 0.2	7.0 ± 0.7	42.3± 1.5	23.7 ± 0.4	2.7± 0.4	1.2 ± 0.4	1.0 ± 0.3	26.8	44.6	28.6
	September 2003	2.1 ± 0.4	18.1± 0.3	1.8 ± 0.4	6.8 ± 0.4	39.8± 0.8	25.3 ± 0.7	3.4± 0.3	1.9 ± 0.4	0.8 ± 0.3	27.0	41.6	31.4
	October 2004	1.3 ± 0.4	17.6± 0.5	1.7 ± 0.7	6.6 ± 0.5	43.9± 0.7	24.3 ± 0.6	2.4± 0.3	1.2 ± 0.3	1.0 ± 0.2	25.5	45.6	28.9
C. *superba* Cham.	September 2002	1.4 ± 0.5	17.3 ± 0.6	2.3 ± 0.5	4.7 ± 0.5	44.3± 0.8	21.8± 0.4	2.9 ± 0.2	2.8 ± 0.4	2.5 ± 0.5	23.4	46.6	30.0
	September 2003	1.3 ± 0.4	16.7 ± 0.5	1.9 ± 0.5	5.6 ± 0.8	46.9 ± 1.3	20.3 ± 1.1	2.2 ± 0.2	3.6 ± 0.5	1.5 ± 0.4	23.6	48.8	27.6
	October 2004	1.4 ± 0.4	17.9 ± 0.8	2.6 ± 0.6	4.8 ± 0.7	45.9 ± 0.8	20.2 ± 0.8	3.1 ± 0.2	2.4 ± 0.5	1.7 ± 0.5	24.1	48.5	27.4
C. *trichotoma* Vell.	June 2002	2.3 ± 0.5	15.1± 0.2	1.8 ± 0.5	8.9 ± 0.9	46.2 ± 0.9	20.4 ± 0.5	2.3 ± 0.4	—	3.0 ± 0.5	26.3	48.0	25.7
	June 2003	1.7 ± 0.6	16.3± 0.5	1.2 ± 0.8	7.1 ± 0.5	46.8 ± 1.5	20.5 ± 0.8	3.3 ± 0.4	—	3.1 ± 0.5	25.1	48.0	26.9
	June 2004	1.4 ± 0.6	18.5± 0.5	1.1 ± 0.5	7.8 ± 0.9	47.0 ± 1.2	17.9 ± 0.7	3.2 ± 0.5	—	3.1 ± 0.4	27.7	48.1	24.2

by our group, GLA was found in seeds of *C. ecalyculata*, *C. myxa,* and *C. sellowiana* collected in Brazil in 2001, ranging from 0.6% to 2.5% of total FA (11). These results suggest that differences in place and time of collection might significantly influence the FA composition of the seeds, although not as much as genetic differences. More detailed studies as to the conditions that result in higher contents of specific FA should be carried out if these seeds are used commercially for the extraction of GLA or other FA.

The concentrations of linoleic, alpha-linolenic, and stearidonic acids and GLA have been indicated as having special taxonomic importance within the Boraginaceae family (12,13,27). Nine species of Boraginaceae from Mongolia (Central Asia) analyzed by Tsevegüren and Aitzetmüller (28) contained GLA (ranging from 6.6% to 13.0%) and stearidonic acid (ranging from 2.4% to 21.4%). *Borago officinalis* L. was found to contain 18.5–24.5% of GLA, but only 0.2– 0.3% of stearidonic acid (12). Another species of Boraginaceae worthy of mention is the plant known in Brazil as confrey (*Symphytum officinale*), as it contains 26–27% of GLA (16,29).

As a previous study indicated, Brazilian *Cordia* species are sources of GLA, a biologically important FA, and five of the six species tested contained GLA in different proportions. The presence of GLA was determined in each class of lipids, and it was found to be predominant in the NL. This is, to our knowledge, the first study on the classes of lipids found in mature seeds of species of *Cordia* found in Brazil.

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