SHORT REPORT

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Variability in Essential Oil Composition of *Croton* Species with Occurrence in the Eastern Brazilian Amazon

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Abstract: The air-dried aerial parts of *Croton campestris*, *C. chaetocalyx*, *C. eriocladus*, and *C. glandulosus*, with occurrence in the Eastern Brazilian Amazon, yielded essential oils, and their volatile constituents were analyzed by GC and GC-MS. Sesquiterpenes, both hydrocarbons and oxygenated, were the most highly represented classes in the oils: the former ranging from 55.3% to 85.1%, and the latter varying from 7.2% to 33.2%. The oils were separated into two groups using hierarchical cluster analysis whose main constituents were β-caryophyllene, germacrene D, γ-elemene, β-elemene, α-humulene and δ-elemene (Group A, *C. campestris* and *C. eriocladus*); and spathulenol, bicyclogermacrene, δ-elemene, germacrene D, β-caryophyllene and δ-cadinene (Group B, *C. chaetocalyx* and *C. glandulosus*). Percentage of sesquiterpene hydrocarbons was higher in Group A (83-85%) than in Group B (55-63%). However, regarding the oxygenated sesquiterpenes, it was reversed, being bigger in Group B (28-33%) than in Group A (7-8%). Percentage of similarity in Group A was 92% and in Group B it was 86%. These chemotaxonomic results showed a significant contribution for the better botanical knowledge of these four *Croton* species occurring in North Brazil.

Keywords: *Croton* spp; Euphorbiaceae; essential oil composition; sesquiterpene compounds; hierarchical cluster analysis.

1. Plant Source

Croton is a genus of Euphorbiaceae comprising about 1200 species widespread in Africa, Asia and South America as sub-shrubs or shrubs and less often trees [1]. Many *Croton* species are used in the traditional medicine of these continents, especially to treat cancer, diabetes, malaria, ulcers, among other diseases [2]. *Croton campestris* A. St.-Hil., known as "velame" [syn. *Croton laetifolius* Baill., *Oxydectes campestris* Kuntze], *Croton chaetocalyx* Müll. Arg. [syn. *Oxydectes chaetocalyx* Kuntze], *Croton eriocladus* Müll. Arg. [syn. *Croton pedicellatus* H.B.K.] and *Croton glandulosus* Müll. Arg. [syn. *Oxydectes glandulosa* Kuntze, *Astraea glandulifera* Klotzsch ex Wawra, *Decanium glandulosum* (L.) Raf. and *Geiseleria glanduosa* (L.) Klotzsch] are perennial species growing in the Brazilian

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biomes known as “cerrado” and “campo rupestre” (savannas and rocky fields), from North to Midwest Brazil [3].

The aim of this report was analyzing the oils composition of the aerial parts (leaf and thin stem) from *Croton campestris*, *C. chaetocalyx*, *C. eriocladus*, and *C. glandulosus* occurring in the Eastern Brazilian Amazon and, in addition, hierarchical cluster analysis was also performed based on the identification of its main constituents.

2. Previous Studies

Some *Croton* barks, when slashed, releases a blood-red sap, known as Dragon´s blood, widely used in traditional medicine to treat and accelerate wound healing [4,5]. *Croton* is also well known for its aromatic character, and more than thirty essential oils have been reported [2]. The main terpenoid compounds frequently found in the *Croton* oils are linalool, 1,8-cineole, β-elemene, α-copaene, germacrene D, epi-α-cadinol, cubenol, epi-cubenol, (*E*)-caryophyllene, selin-11-en-4α-ol, caryophyllene oxide, spathulenol and α-, β- and γ-eudesmol [6]. The volatile components of root bark from *C. campestris* and the leaf oil composition and foliar epicuticular alkanes from *C. glandulosus* were previously reported [7-9].

3. Present Study

The aim of this work was analyzing the oils composition of aerial parts (leaf and thin stem) from *Croton campestris, C. chaetocalyx, C. eriocladus,* and *C. glandulosus* occurring in the Brazilian Eastern Amazon. In addition, the hierarchical cluster analysis was also performed based on the identification of its main constituents.

The samples of *C. campestris* (MG 200165) and *C. glandulosus* (voucher MG 200125) were collected in the municipality of São Geraldo do Araguaia, Pará state, Brazil, February 2011. *Croton chaetocalyx* (voucher MG 200195) and *C. eriocladus* (voucher MG 200198) were sampled in the municipality of Mirador, Maranhão state, Brazil, March 2011. The specimens were identified by Dr. Ricardo Secco, a Euphorbiaceae specialist of the Emílio Goeldi Museum, Belém city, Pará state, Brazil, and then deposited in the Herbarium Murça Pires, existing at the same institution. The aerial parts of the four *Croton* species were air-dried, ground, and submitted to hydrodistillation (100g, 3 h), using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate, and their percentage contents were calculated on the basis of the plant dry weight. The moisture contents of the samples were calculated after phase separation in a Dean-Stark trap (5g, 60 min) using toluene.

The analysis of the oils was carried on THERMO DSQ II GC-MS and GC/FID Focus instruments, under conditions previously described [10]. The retention index was calculated for all the volatiles constituents using an *n*-alkane homologous series [11]. Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds previously analyzed and stored in the data system, using commercial libraries containing retention indices and mass spectra of volatile compounds commonly found in essential oils [12,13].

Cluster analysis was used to classify and group the essential oils according to their main volatile constituents. Complete linkage and absolute correlation coefficient distance were selected as measures of similarity. For the grouping of the oil samples, the agglomerative and hierarchical method was applied. All data were statistically analyzed using the MINITAB 14.0 software.

The aerial parts (leaf and thin stem) of *C. campestris*, *C. chaetocalyx*, *C. eriocladus*, and *C. glandulosus* provided oil yields of 0.5%, 1.1%, 0.6% and 0.7%, respectively, and their volatile constituents were analyzed by GC and GC-MS. In total, ninety-nine components were identified in the oils from *C. campestris*, *C. chaetocalyx*, *C. eriocladus* and *C. glandulosus*, comprising more than 93% of the total composition, which was listed in Table 1. The sesquiterpenes, both hydrocarbons and oxygenated, were the most highly represented classes, the former ranging from 55.3% to 85.1% and the latter varying from 7.2% to 33.2% (Table 2). The monoterpenes, hydrocarbons and oxygenated, were negligently represented in the oils, ranging from 0.1% to 3.3%. With a percentage above 4%, the main compounds found in the oil of *C. campestris* were β-caryophyllene (23.0%), γ-elemene (13.9%), germacrene D (13.7%), β-elemene (7.1%), δ-elemene (6.0%) and bicyclogermacrene (4.7%); in the oil of *C. chaetocalyx* were bicyclogermacrene (13.9%), δ-elemene (13.5%), germacrene D (9.3%), spathulenol (9.0%), δ-cadinene (8.0%) and β-caryophyllene (7.1%); in the oil of *C. eriocladus* were β-caryophyllene (24.1%), germacrene D (17.9%), α-humulene (6.2%), bicyclogermacrene (5.2%) and δ-elemene (5.0%); and in the oil of *C. glandulosus* were spathulenol (19.7%), bicyclogermacrene (9.6%), β-caryophyllene (8.9%), δ-elemene (8.8%), β-elemene (4.7%) and γ-elemene (4.6%).

In order to differentiate between the analyzed *Croton* species, a hierarchical cluster analysis using the compositional profile of the major constituents (above 4%) has been carried out, and the Figure 1 shows the resulting dendrogram. *Croton* oils were separated in two groups (A and B) whose composition is summarized in Table 3. The division of groups showed a correlation with the hydrocarbons and oxygenated sesquiterpenes, which as previously mentioned, are the most representative classes. The following groups have been defined: Group A (composed by *C. campestris* and *C. eriocladus*) with β-caryophyllene (23.0-24.1%), germacrene D (13.7-17.9%), γ-elemene (0-13.9%), β-elemene (3.0-7.1%), α-humulene (2.7-6.2%), δ-elemene (5.0-6.0%), bicyclogermacrene (4.7-5.2%), δ-cadinene (1.6-1.7%) and spathulenol (2.1-2.3%); Group B (composed by *C. chaetocalyx* and *C. glandulosus*) with spathulenol (9.0-19.7%), bicyclogermacrene (9.6-13.9%), δ-elemene (8.8-13.5%), germacrene D (1.6-9.3%), β-caryophyllene (7.1-8.9%), δ-cadinene (1.8-8.0%), β-elemene (0.9-4.7%), γ-elemene (0-4.6%) and α-humulene (1.3-2.4%). The percentage of sesquiterpene hydrocarbons was higher in Group A (83-85%) than in Group B (55-63%). However, regarding the oxygenated sesquiterpenes, it was reversed, being higher in Group B (28-33%) than in Group A (7-8%). The percentage of similarity in Group A was 92% and in Group B it was 86%.

Previously, spathulenol, borneol, caryophyllene oxide, γ-eudesmol and β-caryophyllene were identified as the main volatile components in a root bark oil of *C. campestris*, as well as, β-caryophyllene and γ-elemene in a leaves oil of *C. glandulosus* [7,8]. As shown above, in the present study the oils of leaves and thin stems from C. *campestris* and *C. glandulosus* showed distinct chromatographic profiles. One should take into account not only the use of different parts of the plants, but also the environmental issue because they were sampled at very different places. Therefore. presenting two different chemical profiles. In any case, these oils fall within the terpenoid class, as already observed for the *Croton* species [6]. The oil compositions of *C. chaetocalyx* and *C. eriocladus* were not previously reported.

In conclusion, the four analyzed *Croton* species were divided into two groups based on hierarchical cluster analysis using the compositional profile of the major constituents, where Group A was composed of *C. campestris* and *C. eriocladus* and Group B by the *C. chaetocalyx* and *C. glandulosus*. Sesquiterpene hydrocarbons were higher in Group A (83-85%) than in Group B (55-63%) while the oxygenated sesquiterpenes predominated in Group B (28-33%) when compared with Group A (7-8%). These chemotaxonomic results have presented a significant contribution for the better botanical knowledge of the *Croton* species occurring in the Brazilian Amazon.

Table 2. Percentage of monoterpenes and sesquiterpenes.

Constituents	*C. campestris*	*C. chaetocalyx*	*C. eriocladus*	*C. glandulosus*
Monoterpene hydrocarbons	3.3	0.9	0.5	0.5
Oxygenated monoterpenes	1.3	0.1	0.2	1.0
Sesquiterpene hydrocarbons	83.0	63.4	85.1	55.3
Oxygenated sesquiterpenes	7.2	27.6	7.7	33.2
Other (phenylpropanoids)				1.8
Total	**94.8**	**92.0**	**93.5**	**91.8**

Table 1. Percentage composition of the oils of leaves and thin stems from *Croton* samples.

Constituents	RI*	*Ccamp*	*Cchae*	*Cerio*	*Cglan*	Constituents	RI*	*Ccamp*	*Cchae*	*Cerio*	*Cglan*
α-pinene	934	0.1	0.3	0.1	0.1	viridiflorene	1497		0.1		
camphene	948			0.1	0.1	**bicyclogermacrene**	1500	**4.7**	**13.9**	**5.2**	**9.6**
β-pinene	986	0.1	0.3			β-himachalene	1501			0.4	
myrcene	990	0.4		0.2		α-muurolene	1502	1.2		1.1	0.1
p-cymene	1025				0.1	β-bisabolene	1506				0.8
limonene	1026	1.9			0.1	γ-cadinene	1513	0.3	0.7	0.7	0.2
1,8-cineole	1032		0.1			cubebol	1514		0.2	0.2	1.0
(*E*)-β-ocimene	1045		0.3	0.1	0.1	β-sesquiphellandrene	1523	0.2		0.4	0.1
γ-terpinene	1056	0.7				**δ-cadinene**	1524	1.6	**8.0**	1.7	1.8
terpinolene	1088	0.1				*trans*-calamenene	1525	0.4			0.5
linalool	1095	0.3		0.1	0.1	(E)-γ-bisabolene	1531			0.5	0.1
camphor	1146	0.1				γ-cuprenene	1533			0.1	
pinocarvone	1164					*trans*-cadina-1(2),4-diene	1534		0.2	0.1	0.2
borneol	1165			0.1		α-cadinene	1539	0.8	0.1	0.1	0.2
terpinen-4-ol	1174	0.1				*cis*-sabinene hydrate	1542			0.3	
α-terpineol	1187	0.1			0.1	α-calacorene	1546		0.3	0.2	0.3
bornyl acetate	1289				0.8	selina-3,7(11)-diene	1548				0.8
thymol	1290	0.6				elemol	1549	0.1	0.1		
carvacrol	1297	0.1				*trans*-dauca-4(11),7-diene	1557				0.6
δ-elemene	1335	**6.0**	**13.5**	**5.0**	**8.8**	germacrene B	1560	0.2			0.3
α-cubebene	1348	0.2	0.1	0.1	0.1	(*E*)-nerolidol	1563	0.2			
α-ylangene	1373	0.1	0.1	0.1	0.3	β-calacorene	1566			0.2	
α-copaene	1376	2.9	1.6	2.1	1.5	**spathulenol**	1577	2.3	**9.0**	2.1	**19.7**
β-bourbonene	1388	0.5	0.3	1.3	0.5	caryophyllene oxide	1582	0.6	0.9	1.0	
β-elemene	1390	**7.1**	0.9	3.0	**4.7**	**globulol**	1587	0.3	**4.3**	0.8	0.4
cyperene	1399		0.1	0.5	1.2	viridiflorol	1592		1.9	0.3	0.6
sibirene	1401		0.2			rosifoliol	1600		1.0		
longifolene	1405		0.1			β-atlantol	1609	0.7			
cis-α-bergamotene	1412			0.4		humulene epoxide II	1609			0.6	1.6
β-caryophyllene	1416	**23.0**	**7.1**	**24.1**	**8.9**	junenol	1617		0.2		0.4
β-cedrene	1417			0.1		1,10-di-epi-cubenol	1619		0.5	0.5	
β-copaene	1430		1.3			dillapiole	1621				0.2
β-gurjunene	1432		0.1	2.9		1-*epi*-cubenol	1629		0,2		0.4
trans-α-bergamotene	1433	0.1		2.6		muurola-4,10(14)-dien1β-ol	1631				2.2
γ-elemene	1435	**13.9**			**4.6**	*epi*-α-cadinol	1639	0.3	2.7	0.5	0.4
α-guaiene	1439				0.1	caryophylla-4(12),8(13)-dien-5α-ol	1640				0.5
aromadendrene	1440	0.1	0.7	0.4	0.1	*epi*-α-muurolol	1643	0.4	0.9	0.4	0.4
6,9-guaiadiene	1441			1.2		α-muurolol	1645	0.3	0.6	0.3	0.4
(*Z*)-β-farnesene	1442					*cis*-guaia-3,9-dien-11-ol	1649				0.2
cis-muurola-3,5-diene	1448	1.5	0.6	1.2		β-eudesmol	1651		1.5		
trans-muurola-3,5-diene	1454		0.7			α-eudesmol	1653		1.5		
α-humulene	1455	2.7	1.3	**6.2**	2.4	α-cadinol	1654	1.0	2.1	1.0	1.7
allo-aromadendrene	1462	1.0	1.3	1.3	1.2	selin-11-en-4α-ol	1660	0.9	0.3	0.2	1.2
cis-cadina-1(6),4-diene	1463			0.3		(*E*)-asarone	1675				1.6
trans-cadina-1(6),4-diene	1473			0.1		germacra-4(15),5,10(14)-trien-1α-ol	1685				0.4
γ-gurjunene	1475				0.1	eudesm-7(11)-en-4-ol	1699				0.4
γ-muurolene	1479	0.7		0.7	1.6	isobicyclogermacrenal	1733				0.4
germacrene D	1484	**13.7**	**9.3**	**17.9**	1.6	2α-hydroxy- amorpha-4,7(11)-diene	1775				0.3
aristolochene	1488				0.3	(2*E*,6*E*)-methylfarsenoate	1783				1.6
β-selinene	1489	0.2	0.5	2.3	0.5						

*Retention index (DB-5ms capillary column); *Ccamp = Croton campestris*, *Cchae = Croton chaeticalyx*, *Cerio = Croton eriocladus*, *Cglan = Croton glandulosus*.

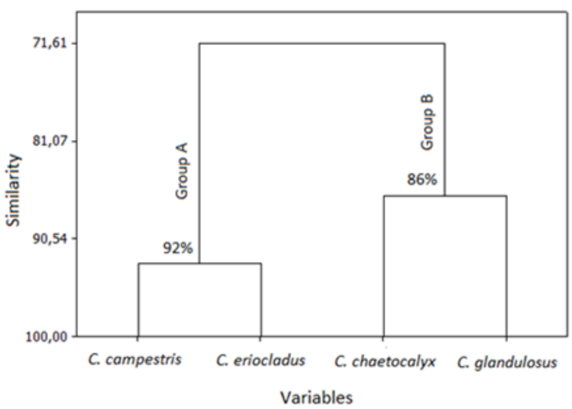

Figure 1. Dendrogram resulting from cluster analysis of the oils of *Croton* species.

Table 3. Cluster analysis for the main sesquiterpenes identified in Croton oils.

Components (%)	Group A	Group B
	C. campestris *C. eriocladus*	*C. chaetocalyx* *C. glandulosus*
Sesquiterpenes hydrocarbons	**83 - 85**	**55 - 63**
δ-elemene	5 - 6	≈ 9- ≈ 14
β-elemene	3 - ≈ 7	≈ 1 - ≈ 5
β-caryophyllene	23 - 24	≈ 7 - ≈ 9
γ-elemene	0 - ≈ 14	0 - ≈ 5
α-humulene	≈ 3 - 6	1 - 2
germacrene D	≈ 14 - ≈ 18	≈ 2 - 9
bicyclogermacrene	≈ 5	≈ 10 - ≈ 14
δ-cadinene	≈ 2	≈ 2 - 8
Oxygenated sesquiterpenes	**7 - 8**	**≈ 28 - 33**
spathulenol	≈ 2	9 - ≈ 20

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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