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. © 2015 ACG Publications. All rights reserved.

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, Astraera glandulifera Klotzsch ex Wawra, Decanum glandulosum (L.) Raf. and Geiseleria glanduosa (L.) Klotzsch] are perennial species growing in the Brazilian...
biosomes 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 Araguai, 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 volatile compounds 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>
<thead>
<tr>
<th>Constituents</th>
<th><em>C. campestris</em></th>
<th><em>C. chaetocalyx</em></th>
<th><em>C. eriocladus</em></th>
<th><em>C. glandulosus</em></th>
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<td>0.2</td>
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<td>85.1</td>
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<td>27.6</td>
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<td>Other (phenylpropanoids)</td>
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<td><strong>94.8</strong></td>
<td><strong>92.0</strong></td>
<td><strong>93.5</strong></td>
<td><strong>91.8</strong></td>
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Table 1. Percentage composition of the leaves of thin stems from *Croton* samples.

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<th>Constituents</th>
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<th>Comp</th>
<th>Chue</th>
<th>Cerio</th>
<th>Cglan</th>
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</table>

Retention index (DB-5ms capillary column); Comp = Croton campestris, Chue = Croton characalyx, Cerio = Croton eriocladus, Cglan = Croton glandulatus.

Table 3. Cluster analysis for the main sesquiterpenes identified in Croton oils. Component (%) Group A Group B

<table>
<thead>
<tr>
<th>Component</th>
<th>Group A</th>
<th>Group B</th>
</tr>
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<tr>
<td></td>
<td>C. campestris</td>
<td>C. characalyx</td>
</tr>
<tr>
<td></td>
<td>C. eriocladus</td>
<td>C. glandulatus</td>
</tr>
<tr>
<td>Sesquiterpenes hydrocarbons</td>
<td>83.85</td>
<td>55.63</td>
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<tr>
<td>β-elemene</td>
<td>5 - 6</td>
<td>7 - 8</td>
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<tr>
<td>β-caryophyllene</td>
<td>23 - 24</td>
<td>7 - 9</td>
</tr>
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<td>γ-elemene</td>
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<td>Oxygenated sesquiterpenes</td>
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<td>14 - 20</td>
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Figure 1. Dendrogram resulting from cluster analysis of the oils of *Croton* species.
Acknowledgements

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Supporting Information

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

References