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Volatile Constituents of Two *Croton* Species from Caatinga Biome of Pernambuco – Brasil

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Abstract: Leaf and stem essential oils from *Croton pulegioides* Müll.Arg. and *C. rhamnifolius* var. *heliotropiifolius* (Kunth) Müll.Arg were obtained by hydrodistillation and analyzed using gas chromatography and gas chromatography-mass spectrometry. The composition of the oils from the two *Croton* species was very different. The main components of the *C. pulegioides* oils were 1,8-cineole (15.86± 0.23% in leaf), p-cymene (14.40± 0.01% in leaf), camphor (13.28%± 0.12% in leaf) and α-humulene (12.98± 0.22% in leaf), α-calacorene (12.95± 0.45% in stem), *cis*-isolongifolane (8.94±0.54% in stem) and juniper camphor (6.44±0.45% in stem). The main components of the *C. rhamnifolius* var. *heliotropiifolius* oils were β-caryophyllene (20.82±0.48% in leaf), spathulenol (16.37±0.56% in leaf) and β-elemene (17.28±0.06% in stem) and guaiol (18.38±0.84% in stem). Phenylpropanoids common to *Croton* species were only found in *C. rhamnifolius* var. *heliopropiifolius* oils at percentages below 5%. This is the first report of the essential oil constituents of *C. pulegioides* and *C. rhamnifolius* var. *heliotropiifolius* from the Caatinga biome of the state of Pernambuco (Northeastern Brazil).
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Keywords: *Caatinga biome; Croton pulegioide; Croton rhamnifolius var. heliotropiifolius;* essential oil composition.

1. Plant Source

Croton L. belongs to the family Euphorbiaceae and is one of the largest genera of flowering plants, with nearly 1300 species of herbs, shrubs and trees. In Brazil, about 300 species are widely distributed throughout semiarid regions, beaches and tropical rainforests [1]. Plants from this genus are among the best sources of bioactive compounds from the family Euphorbiaceae and are reported to be rich in diterpenes [2] and alkaloids [3] and are characterized by their essential oil production [4-9].

The major center of diversity of the genus *Croton* in the state of Pernambuco (PE) (Northeastern Brazil) is the Caatinga biome, which is the characteristic flora of the semi-arid region. These forests are rich in aromatic bushes, vines, herbs and trees that are well adapted to the drastic climatic conditions. The species *C. pulegioides* and *C. rhamnifolius* var. *heliotropiifolius* are among the abundant aromatic plants in the Caatinga vegetation in the state of PE. These plants are locally known as "*velame*" or "*marmeleiro*" and are used as folk remedies for the treatment of wounds, inflammation and cancer [10]. Leaves and stems of *C. pulegioides*, *C. rhamnifolius* var. *heliotropiifolius* were collected, in july 2007, in the Caatinga region in the municipality of Serra Talhada in PE, Brazil. The plants were identified by Dr. Maria de Fátima de Araújo Lucena from the

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Centro de Saúde e Tecnologia Rural-Unidade Acadêmica de Ciencias Biologicas, Universidade Federal de Campina Grande. Vouchers species were deposited in the Herbário Geraldo Mariz, da Universidade Federal de Peranambuco, under the numbers: 33456 = *C. pulegioides* and 30941 = *C. rhamnifolius* var. *heliotropiifolius*.

1. Previous Studies

To the best of our knowledge, no phytochemical studies have previously been reported on this two *Croton* species occurring in PE.

2. Present Study

Fresh leaves and stem were collected separately from the trees under investigation, subdivided in three portions of 100g each, chopped and then subjected to hydrodistillation for two hours in a Clevenger-type apparatus. Oil layers obtained were separated, dried over anhydrous sodium sulphate, weighed, stored in hermetically sealed glass containers and kept under refrigeration at + 5 ºC before analysis. The yield calculations were realized by the relation of volatile oil volume and the mass of fresh plant material used for extraction. To yields determination of each sample, all experiments were carried out in triplicate. Quantitative and qualitative analysis of the oils were performed by GC and GC/MS, respectively [11,12]. The hydrodistillation of different parts of *C. pulegioides* and *C. rhamnifolius* var. *heliotropiifolius* yielded yellowish, pleasant-smelling oils. The greatest yield was obtained from *C. pulegioides* (leaf = 0.64±0.06% and stem = 0.75±0.03%), which was significantly different from the of *C. rhamnifolius* var. *heliotropiifolius* (leaf = 0.12±0.01% and stem = 0.01±0.02%). The volatile constituents identified in these oils are listed in Table 1.

Table 1. Percentage compositions of the essential oils from *Croton* species

Compond	RI.[a]	RI.[b]	CPUL		CRHA		Method of identification
			Leaf	Stem	Leaf	Stem	
Tricyclene	929	926	0,10±0.00	-	-	-	RI, MS
α-Pinene	936	939	0,41±0.02	-	-	-	RI, MS, CI
α-Fenchene	949	951	0,17±0.07	-	-	-	RI, MS
Sabinene	975	976	0,20±0.03	-	-	-	RI, MS
β-Pinene	978	980	0,15±0.05	-	-	-	RI, MS, CI
Myrcene	994	991	1,82±0.12	-	-	-	RI, MS
α-Phellandrene	1006	1005	1,49±0.14	-	-	-	RI, MS
α-Terpinene	1019	1018	0,33±0.00	-	-	-	RI, MS, CI
o-Cymene	1024	1022	-	-	1,23±0.02	2,10±0.12	RI, MS
p-Cymene	1028	1026	14,40±0.01	-	-	-	RI, MS, CI
1,8-Cineole	1035	1033	15,56±0.23	-	-	-	RI, MS, CI
γ-Terpinene	1063	1062	0,75±0.36	-	-	-	RI, MS
Linalool	1097	1098	3,67±0.15	0,73±0.01	-	-	RI, MS, CI
Camphor	1133	1143	13,28±0.12	1,13±0.33	-	-	RI, MS
Isoborneol	1154	1156	1,09±0.65	-	0.21±0.01	3,34±0.21	RI, MS
Terpin-4-ol	1175	1177	3,24±0.45	0,51±0.02	-	-	RI, MS
α-Terpineol	1190	1189	5,86±0.88	1,58±0.01	-	-	RI, MS, CI
Linalyl acetate	1260	1257	1,50±0.11	-	-	-	RI, MS
Bornyl acetate	1287	1285	-	-	0.31±0.04	1,91±0.01	RI, MS
Isoledene	1376	1373	-	0,74±0.01	-	-	RI, MS
α-Copaene	1379	1376	-	1,58±0.08	-	-	RI, MS
β-Elemene	1395	1391	-	1,44±0.06	6,81±018	17,28±0.06	RI, MS
Cyperene	1400	1398	-	-	0.52±0.02	1,36±0.04	RI, MS

α-Gurjunene	1411	1409	-	3,27±0.12	-	-	RI, MS
β-Caryophyllene	1420	1418	-	-	20,82±0.48	8,05±0.11	RI, MS, CI
β-Gurjunene	1434	1432	-	3.04±0.19	-	-	RI, MS
γ-Elemene	1436	1433	-	2,59±0.07	0,93±0.04	0.59±0.11	RI, MS
Aromadendrene	1442	1439	5,22±0.44	-	-	-	RI, MS
α-neo-Clovene	1450	1454	-	0,47±0.01	-	-	RI, MS
α-Humulene	1456	1454	12,98±0.22	-	3,92±0.12	1,87±0.21	RI, MS, CI
α-Patchoulene	1458	1456	-	1,07±0.01	-	-	RI, MS
9-epi-(*E*)-Caryophyllene	1469	1467	-	1,40±0.05	-	-	RI, MS
α-Terpinyl isobutyrate	1473	1471	-	4,75±0.88	-	-	RI, MS
γ-Gurjunene	1476	1473	-	2,40±0.59	-	-	RI, MS
cis-β-Guaiene	1490	1490	-	6,48±0.05	-	-	RI, MS
Bicyclogermacrene	1493	1494	-	-	6,59±0.08	2,43±0.23	RI, MS
β-Himachalene	1500	1499	-	3,36±0.07	-	-	RI, MS
trans-β-Guaiene	1505	1500	-	3,44±0.88	-	-	RI, MS
β-Bisabolene	1508	1509	-	-	0,93±0.06	1,68±0.35	RI, MS
γ-Cadinene	1515	1513	-	1,49±0.95	-	-	RI, MS
Cubebol	1517	1514	-	5,49±0.99	-	-	RI, MS
δ-Cadinene	1526	1524	-	1,92±0.65	-	1,16±0.41	RI, MS
β-Sesquiphellandrene	1527	1524	1,05±0.55	0,73±0.22	-	-	RI, MS
(*Z*)-Nerolidol	1536	1534	-	-	0,70±0.06	0.35±0.04	RI, MS, CI
α-Cadinene	1540	1538	-	1,58±0.33	-	-	RI, MS
α-Calacorene	1545	1542	-	12,95±0.45	-	-	RI, MS
Elemol	1551	1549	-	-	0,83±0.04	3,01±0.06	RI, MS
Elemicine	1556	1554	-	-	0.42±0.00	1,01±0.01	RI, MS
Germacrene B	1559	1556	-	-	9,33±0.09	2,83±0.045	RI, MS
β-Calacorene	1566	1563	-	6,51±0.33	-	-	RI, MS
Spathulenol	1577	1576	-	-	16,37±0.56	1,43±0.08	RI, MS
Caryophyllene oxide	1582	1581	-	-	8,34±0.64	2,06±0.88	RI, MS, CI
dihydro-Ar-Turmerone	1595	1591	-	6,08±0.78	-	-	RI, MS
Guaiol	1596	1595	-	-	4,99±0.31	18,38±0.84	RI, MS
cis-Isolongifolanone	1605	1606	3,91±0.54	8,94±0.89	-	-	RI, MS
β-Oplopenone	1609	1606	4,92±0.21	-	-	-	RI, MS
β-Himachalene oxide	1618	1610	-	-	0,99±0.02	0.58±0.21	RI, MS
3-*iso*-Thujopsanone	1636	1637	-	3,68±0.56	-	-	RI, MS
epi-α-Cadinol	1642	1640	0,34±0.05	-	-	-	RI, MS
epi-α-Muurolol	1644	1641	-	-	-	1,53±0.22	RI, MS
Cubenol	1645	1642	0,37±0.01	-	-	2,49±0.45	RI, MS
α-Muurolol	1646	1645	-	-	-	0,91±0.01	RI, MS
Himachalol	1648	1647	-	-	1,57±0.31	0.50±0.00	RI, MS
β-Eudesmol	1650	1649	-	-	1,61±0.25	2,62±0.02	RI, MS
Valerianol	1654	1655	3,02±0.65	-	3,66±0.51	10,62±0.84	RI, MS
Bulnesol	1664	1666	1,63±0.21	-	1,42±0.61	5,84±0.07	RI, MS
Foeniculin	1675	1677	-	-	1,99±0.46	3,26±0.08	RI, MS
Juniper Camphor	1692	1691	-	6,44±0.45	-	-	RI, MS
MH			35.38±0.20	3.95±0.02	1.80±0.02	2.10±0.00	
OM			28.64±0.14	-	0.52±0.01	5.25±0.18	
SH			19.25±0.17	56.46±0.21	49.33±0.02	37.25±0.22	
OS			14.20±0.11	35.38±0.19	40.48±0.13	50.34±0.12	
Phenylpropanoid			-	-	2.41±0.09	4.27±0.02	
Total			97.47±0.25	95.79±0.88	94.48±0.04	99.21±0.06	

[a]Retention indices calculated from retention times in relation to those of a series of n-alkanes on a 30m DB-5 capillary column. [b]Linear retention indices from the literature. CPUL = *Croton pulegioides*, CRHA = *Croton rhamnifolius* var. *heliotropiifolius*. RI = retention index, MS = mass spectrum, CI = co-injection with authentic standards. MH and SH = Mono and Sesquiterpenes Hycrocarbons, OM and OS = Oxygenated Mono and Sesquiterpenes.

All the *Croton* oils were made up of mono and sesquiterpenes with a very diverse composition. Phenylpropanoids were only found in *C. rhamnifolius* var. *heliotropiifolius* oils, at low percentages. Fifty-four components were identified in the *C. pulegioides* oil, representing approximately 97.47±0.13% and 95.79±0.88% of the leaf and stem oils, respectively. Among these components, only linalool, camphor, terpin-4-ol, α-terpineol, β-sesquiphellandrene and *cis*-isolongifolanone are found simultaneously in the leaf and stem oils. Monoterpenoids constituted 64.02% of the leaf oil, with the most abundant component being 1,8-cineole (15.56±0.23%), followed by *p*-cymene (14,40±0.01%) and camphor (13.28±0.12%). Other monoterpenonoids that were present in appreciable amounts were α-terpineol (5.86±0.88%), linalool (3.67±0.15%) and terpin-4-ol (3.24±0.45%). Other components of this chemical group were present at amounts less than 1%, except for Myrcene (1.82±0.12%), α-phelladrene (1.49±0.14%) and isoborneol (1.09±0.65%). Sesquiterpenoids constituted 33.45±0.22% of this oil, with α-humulene (12.98±0.22%) and aromadendrene (5.22±0.44%) the most abundant constituents. A total of 29 substances were identified in the *C. pulegioides* stem oil, accounting for 95.79±0.88% of the oil. Unlike the oil from the leaves, the stem oil was characterized by large amounts of oxygenated sesquiterpenes (91.84±0.01%), with the following as the major constituents: α-calacorene (12.95±0.45%), *cis*-isolongifolanone (8.94±0.89%), β-calacorene (6.51±0.33%), *cis*-β-guaiene (6.48±0.05%), dihydro-ar-turmerone (6.08±0.78%), juniper camphene (6.44±0.45%) and cubenol (5.49±0.99%). The monoterpene fraction was relatively small, accounting for only 3.95±0.02% of the total oil.

These results suggest that the distribution and accumulation of monoterpenes and sesquiterpenes in the *C. pulegioides* oils differ depending on the part of the plant investigated. According to the CG and CG/MS analyses of the leaf and stem oil from *C. rhamnifolius* var. *heliotropiifolius*, the chemical composition of the two oils is qualitatively similar. About 23 and 27 components were identified in the *C. rhamnifolius* var. *heliotropiifolius* leaf and stem oils, comprising 94.48±0.22% and 99.21±0.15 %, respectively. All compounds identified in the leaf oil were found in the stem oil. The principal difference between the two oils was the absence of δ-cadinene, epi-α-muurolol, α-muurolol and cubenol from the leaf oil. Both oils were characterized by high percentages of sesquiterpenes (89.81±0.01% in leaf and 87.59±0.05% in stem), particularly β-caryophyllene (20.82±0.48%), spathulenol (16.37±0.56%) and germacrene B (9.33±0.09%) in the leaf oil and guaiol (18.38±0.84%), β-elemene (17.28±0.06%) and valerianol (10.62±0.84%) in the stem oil. Comparing the Sesquitepenoid fractions in both oils, the leaf oil was dominated by hydrocarbon sesquiterpenes (49.33±0.02%), while the stem oil was rich in oxygenated sesquiterpenes (50.34±0.12%). Phenylpropanoids have been reported to constitute the oil of *Croton* species collected in the state of PE [5] as well as other regions of Brazil [13] and the world, such as Venezuela [14] and Mexico [15]. The results reported here reveal that only *C. rhamnifolius* var. *heliotropiifolius* is made up of phenylpropanoids derivatives. Elemicin and foeniculin accounted for less than 2% and 5% of the leaf and stem oil, respectively. Systematic chemical studies on the essential oils of a number of Brazilian species have been carried out, with the major components reported to be α, β-pinene, linalool or β-caryophyllene [16]. Among these compounds, α- and β-pinene were only found in the leaf oil of *C. pullegioides*, but at amounts of less than 1%. Linalool (3.67±0.15%) was also found in this oil, but not as a main component. The oil from *C. rhamnifolius* var. *heliotropiifolius* revealed a chemical profile that is more characteristic of the genus *Croton*. The presence of β-caryophyllene in significant amounts in the leaf (20.82±0.48%) and stem (8.05±0.11%) oil is in agreement with the proposal put forth by Bracho and Crowle [17] that the occurrence of β-caryophyllene as a major constituent may be a characteristic of *Croton* essential oils.

The oils from two *Croton* species analyzed in the present study seem to be quite different. These results reveal that the variability (differences and/or similarities) in the composition of essential oils depends essentially upon the origin of the samples as well as the influence of geographic circumstances and climate.

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