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Chemical compounds from species of Croton native to South Brazil

Sita Luvangadio Lukoki Vunda[a], Sérgio A. L. Bordignon[a], Eduardo Cassel[b], Rubem M.F. Vargas[b], Gilsane Lino von Poser[a], Miriam Anders Apel*[a]

[a]*Programa de Pós-Graduação em Ciências Farmacêuticas, UFRGS, CEP 90610-000 Porto Alegre, RS, Brazil;*
[b]*PGETEMA – Faculdade de Engenharia, PUCRS, CEP 90619-900 Porto Alegre, RS, Brazil.*
**Corresponding author: miriam.apel@ufrgs.br*

Five species of *Croton* were investigated for the essential oil obtained by hydrodistillation from aerial parts of the plants and analyzed by gas chromatography/mass spectrometry. The essential oil yields from fresh weight ranged from 0.01% to 1%. The essential oil of *C. hilarii* Baill. exhibited the lowest yield (0.01%). Following this, *C. pycnocephalus* Müll.Arg. oil yielded 0.1%, while *C. gnaphalii* Baill. achieved 0.2%. Conversely, *C. calycireduplicatus* Allem and *C. cuchillae-nigrae* Croizat afforded the highest yields, both reaching 1%. The essential oils from the latter two species were rich in monoterpenes (52.5% and 59.9%, respectively), being β-pinene the prevalent compound (32.8% and 40.1%, respectively). In contrast, the essential oils from *C. pycnocephalus* and *C. gnaphalii* were characterized by a substantial content of sesquiterpenes (85.7% and 79.1%, respectively), being bicyclogermacrene (26.4% and 31%) the predominant component. The *C. hilarii* essential oil presented only sesquiterpene in its composition, among which sesquicineole (19.90%) and *epi*-α-bisabolol (16.4%) were the most abundant. The species with high amount of essential oil (*C. calycireduplicatus* and *C. cuchillae-nigrae)* were subjected to supercritical fluid extraction (SFE) at a temperature of 40 °C and different pressions. The fractions were analyzed by GC-MS. The SFE demonstrated pressure-dependent selectivity for compound classes. At 80 bar, sesquiterpenes were predominantly obtained. Increasing pressure led to the progressive disappearance of lower molecular weight terpenes and extraction of triterpenes (e.g., amyrins), and hydrocarbons at higher pressures. Nevertheless, SFE yields were generally low, indicating the conditions used were not optimal for the plants in study.

Keywords: *Croton;* Euphorbiaceae; essential oil; hydrodistillation; supercritical CO_2.

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Introduction

Euphorbiaceae, an important Angiosperm family, is a diverse botanical group encompassing approximately 330 genera and 6300 species globally distributed (1). The species can grow as trees, shrubs, subshrubs, herbs, or lianas. Some of them have a cactus-like appearance, often mistaken for true cacti due to their succulent stems and spines (2). One of the defining botanical characteristics of Euphorbiaceae is the presence of *laticifer* containing white or colored latex which becomes visible when the plant is subjected to mechanical injuries (3,4).

The family includes species of large economic importance such as *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (rubber tree), native to South America, occurring mainly in the Amazon rain Forest, *Ricinus communis* L. (castor oil plant), an African species widely introduced all over the world, and *Manihot esculenta* Crantz (cassava, also known as aipim or macaxeira), among others (5,6). Several members of Euphorbiaceae are worldwide recognized for their diverse array of toxic and medicinal properties, a reflection of the significant chemical diversity inherent within this plant group (7).

The main genera in number of species are *Euphorbia* (around 2000 species) and *Croton* (about 1200 species). *Croton* is particularly diverse in the Americas, especially in Brazil, with 316 species of herbs, shrubs, trees, and sometimes lianas that occur in almost all ecosystems (1).

Croton is characterized by a wealth of bioactive constituents, especially diterpenoids such as clerodanes, phorbol esters, labdanes, kauranes, trachylobanes, and pimarane derivatives. Among these, clerodane diterpenoids are the most extensively distributed, identified in *Croton* species across America, Africa, and Asia. Phorbol esters also show a broad distribution, present in species from Asia, Africa, and America. The other classes of diterpenoids have a more limited distribution. In addition to diterpenes, several *Croton* species contain essential oils. Analyses of the essential oils from some of these species have identified monoterpenoids, sesquiterpenoids, and phenylpropanoids as major constituents (7-10).

The essential oils of *Croton* have been traditionally obtained by *hydrodistillation* using a Clevenger apparatus but, although less frequently, also by supercritical fluid extraction (SFE) (11,12). Diterpenes have been extracted using apolar or moderately polar solvents. Nevertheless, some studies have been reported supercritical fluid extraction as an efficient technique to obtain these compounds (13-15).

With the aim of verifying the efficiency of supercritical extraction in obtaining essential oils, the two richest *Croton* species where subjected to SFE. In addition, to essential oils there was an expectation of finding diterpenes in the SFE extracts of these species.

Supercritical fluid extraction (SFE) is an environmentally friendly technique recognized for its ability to enhance the extraction yield and selectivity of compounds from plant material. A fundamental advantage of the technique is the possibility of modifying parameters such as pressure and temperature, allowing for increased extraction selectivity. Carbon dioxide (CO_2) is the most widely utilized solvent in SFE, primarily because of its low critical temperature (31.1 °C) and pressure (73.8 bar), ease of residual solvent removal (by reducing pressure), low cost, non-flammability, and non-toxicity. Consequently, SFE stands as an eco-friendly technology, employing non-toxic solvents for challenging extractions and significantly reducing processing times compared to conventional methods (16).

In this context, the purpose of this study was to characterize the essential oils from the aerial parts of *C. pycnocephalus* Müll.Arg., *C. hilarii* Baill., *C. gnaphalii* Baill., *C. calycireduplicatus* Allem and *C. cuchillae-nigrae* Croizat obtained by hydrodistillation using gas chromatography coupled to mass spectrometry. The plant material from *C. calycireduplicatus* and *C. cuchillae-nigrae* was also extracted by supercritical CO_2.

Experimental section

Plant Material

The aerial parts of the *Croton* spp. (Figure 1) were collected in the Rio Grande do Sul state, South Brazil, and voucher specimens were deposited in the herbarium of Universidade Federal do Rio Grande do Sul (ICN). The collection data such as location, collection date, voucher and coordinates of the plants that were used as research material are described in Table 1.

GC-MS analyses

For chemical analyses, the samples were diluted in ethyl ether at a ratio 2:100 (v/v). The chemical composition was determined by gas chromatography coupled with mass spectrometry (GC/MS) (Shimadzu QP5000) equipped with a capillary column of fused silica Durabond-DB5 (John Wiley & Sons Scientific, US, 30 m×0.25 mm×0.25 μm). The source temperature, quadrupole and injector were set at 230 °C, 150 ºC and 220 °C, respectively, using a column temperature programming of 60 °C–300 °C at 3 °C/min. Ultrapure helium was used as the carrier gas at 80 kPa and flow of 1 mL/min. Scan time and mass range were 1 sec and m/z 40 - 550 m/z, respectively, with electron energy of 70 eV. One microliter was injected in the split mode ratio of 1:20. The identification of compounds was based on the comparison of retention indices calculated by linear interpolation relative to retention times of a series of *n*-alkanes (C_8 to C_{32}), and their mass spectra with authentic samples and with data collected from the literature (17) or by comparison with mass spectra recorded in the database of NIST 62 and NIST 12 (National Institute of Technology and Standards). The relative amounts of the components were calculated based on GC peak areas by normalization. The percentage areas were determined without using response factors.

Table 1. Collection data of *Croton* species collected in Rio Grande do Sul state.

Species[1]	Local	Coordinates	Collection date	Voucher
Ccaly	Cachoeira do Sul	30S045'16" 52W55'11"	21/07/2022	205500
Ccuch	Barão do Triunfo	30S23'27" 51W44'18"	07/07/2022	205501
Cgna	Barão do Triunfo	30S23'10" 51W44'01"	07/07/2022	203798
Cpyc	Barão do Triunfo	30S25'42" 51W85'73"	11/02/2022	203801
Chil	Santo Antônio da Patrulha	30S22'10" 50W36'22"	17/02/2022	203802

[1] *Ccaly*: *C. calycireduplicatus, Ccuch: C. cuchillae-nigrae, Cgna: C. gnaphalii, Cpyc: C. pycnocephalus, Chil: C. hilarii.*

Supercritical fluid extraction

Extraction with supercritical carbon dioxide was performed in a 0.5 L high-pressure extraction vessel (internal diameter of 5.4 cm), in which the dried and powdered aerial parts of the plants (40 g) were inserted. This extraction vessel is part of a pilot unit, described in detail by (18), which has pressure, temperature and flow controllers. Pressure values were monitored with a Novus 8800021600 digital transducer system, whose accuracy was ± 1.0 bar. Temperature controllers (PT-100) are positioned on the equipment to measure the temperature of the carbon dioxide inlet and inside the extraction vessel. The control software of this unit was developed using the Elipse SCADA platform developed by the company Elipse Software Ltda. The solvent used was supercritical carbon dioxide with a degree of purity equal to 99.9% (Air Products) which flowed with a flow rate of 1 kg h−1 measured by a flowmeter test (Sitraus FC Massflo 2100 - Siemens) with 0.1% accuracy. The flow of the solvent is carried out by a pump (Thar P - 200, USA). In the extraction experiments there was no recycling of carbon dioxide. Using the extraction protocol reported in previous

studies of the research group (19,20), the plant material was subjected to extraction at temperature of 40 °C and under successively increased pressures (80, 150, 200, 250, and 300 bar), collecting the samples of each pressure after 30 min.



Figure 1. *C. pycnocephalus* Müll.Arg. (A), *C. gnaphalii* Baill. (B), *C. cuchillae-nigrae* Croizat (C), *C. hilarii* Baill. (D), *C. calycireduplicatus* Allem (E). Fonte: Sérgio Bordignon.

Results and Discussion

Chemical composition of the essential oils

The initial phase of this study involved the chemical characterization of essential oils derived from five native *Croton* species endemic to South Brazil. To our knowledge, the constituents of their essential oils have not been previously reported in the literature. All essential oil samples were obtained through hydrodistillation.

The essential oil yield, determined on a fresh weight (w/v) basis, ranged from 0.01% to 1%. *Croton hilarii* exhibited the lowest yield (0.01%), followed by *C. pycnocephalus* (0.1%) and *C. gnaphalii* (0.2%). The highest yield (1%) was observed for *C. calycireduplicatus* and *C. cuchillae-nigrae*. Table 2 summarizes the chemical composition of these essential oils, including retention indices and component percentages identified by gas chromatography-mass spectrometry.

Croton species showed a great chemical diversity, among hydrocarbon and oxygenated monoterpenes, hydrocarbon and oxygenated sesquiterpenes and some acids. The essential oil of *C. calycireduplicatus* and *C. cuchillae-nigrae* was characterized by the predominance of monoterpene hydrocarbons being β-pinene (32.8% and 40.1%, respectively), the major constituent, followed by oxygenated sequiterpene, viridiflorol (13.4% e 15.5%, respectively). *Croton pycnocephalus*, *C. hilarii* and *C. gnaphalii* were mainly characterized by sesquiterpenes, in which bicyclogermacrene was the main compound among the sesquiterpenes hydrocarbons (26.4%, 8.2% and 31.0%, respectively) followed by β-caryophyllene (7.4%, 6.4% and 13.9%, respectively). In the oxygenated sesquiterpenes fraction, *C. pycnocephalus* also presented high amount of spathulenol (12.8%) and *iso*-spathulenol (12.1%) and for *C. hilarii*, sesquicineole (19.9%) and *epi*-α-bisabolol (16.4%) were also present in considerable quantity. Hexadecanoic acid was present in appreciable amount (12.3%) in this last mentioned essential oil.

These results are in accordance to those obtained for the essential oils of other species of *Croton*, native to Rio Grande do Sul, investigated by our research group (8). *Croton calycireduplicatus* and C. *cuchillae-nigrae* are similar to *C. ericoides* Baill., whose essential oil is characterized by the presence of mono- and sesquiterpenes, with β-pinene being the major compound (39%). On the other hand, the essential oil of *C. pycnocephalus* and *C. hilarii* were not characterized by the presence of monoterpenes, as well as *C. isabelli* Baill., in which no compound of this group were detected. Finally, *C. gnaphalii*, exhibited a similar profile of that shown by *C. pallidulus* Baill. essential oil, with low amount of monoterpenes.

Table 2. Percentual composition of the essential oils obtained by hydrodistillation from *Croton* species collected in Rio Grande do Sul.

		C. calycireduplicatus		*C. cuchillae-nigrae*		*C. pycnocephalus*	*C. hilarii*	*C. gnaphalii*
Yeld (%)		1.0		1.0		0.1	0.01	0.2
RRI[a]	**Components**	HD	CO_2 extraction 80 bar	HD	CO_2 extraction 80 bar			
				Monoterpene hydrocarbons				
920	α-Thujone	1.9	0.1	0.4	-	-	-	-
925	α-Pinene	3.5	0.4	5.2	-	-	-	-
927	α-Fenchene	0.1	-	-	-	-	-	-
967	β-Pinene	32.8	5.4	40.1	-	-	-	3.5
983	Myrcene	1.0	-	0.7	-	-	-	-
997	α-Phellandrene	0.4	-	1.8	-	-	-	-
1010	α-Terpinene	1.3	-	0.8	-	-	-	-
1017	*p*-Cymene	1.2	1.8	0.3	-	-	-	-
1022	Limonene	1.1	0.2	-	-	-	-	-
1042	*E*-β-Ocimene	-	-	-	-	-	-	0.2
1051	γ-Terpinene	4.1	-	0.6	-	-	-	-
1072	Terpinolene	1.3	-	0.6	-	-	-	-
	Total	48.6	7.9	49.8	0	0	0	3.7
				Oxygenated monoterpenes				
1023	1,8-Cineole	0.4	0.4	6.6	0.3	-	-	-
1096	Linalool	0.8	0.3	1.3	-	0.7	-	5.8
1131	*E*-pinocarveol	0.8	0.7	-	-	-	-	-
1148	Pinocarvone	-	0.1	-	-	-	-	-
1170	Terpinen-4-ol	1.3	1.5	0.5	-	-	-	-
1172	*p*-cimen-4-ol	-	0.1		-	-	-	-
1179	α-Terpineol	0.6	0.3	1.7	0.6	-	-	-
1180	Myrtenal	-	0.1	-	-	-	-	-
1289	Myrtenol	-	0.4	-	-	-	-	-

	Total	3.9	3.9	10.1	0.7	0.7	0	5.8
				Sesquiterpene hydrocarbons				
1321	δ-Elemene	0.7	-	0.7	-	-	-	-
1349	α-Ylangene	-	0.2	-	-	-	-	-
1364	α-Copaene	-	0.6	-	-	-	0.1	0.2
1373	β-Bourbonene	-	1.2	-	-	0.4	0.4	0.5
1374	β-cubebene	-	0.2	-	-	-	-	-
1381	β-Elemene	0.1	-	-	-	-	0.1	0.1
1390	α-Gurjunene	0.1	-	-	-	-	-	-
1407	β-Caryophyllene	2.4	0.7	2.9	0.2	7.4	6.4	13.9
1425	γ-Elemene	-	-	-	-	-	0.3	-
1426	Aromadendrene	0.2	0.3	0.3	3.3	-	-	-
1439	α-Humulene	0.4	0.1	0.6	-	0.9	0.5	2.2
1447	*Allo*-aromadendrene	1.2	2.3	1.0	3.0	1.1	0.6	1.1
1457	9-epi-*E*-caryophyllene	-	0.5	-	-	-	-	-
1461	Viridiflorene	0.6	1.9	-	-	-	-	-
1465	γ-Muurolene	-	-	0.1	0.7	-	-	-
1469	Germacrene D	-	-	0.3	-	2.2	9.1	2.5
1486	Bicyclogermacrene	4.1	-	4.6	-	26.4	8.2	31.0
1489	α-Muurolene	0.5	0.3	4.6	0.3	-	0.2	-
1493	Germacrene A	-	-	-	-	-	0.5	0.2
1500	γ-Cadinene	0.6	-	0.4	2.2	1.1	-	0.7
1508	δ-Amorphene	-	-	-	-	-	-	1.0
1512	δ-Cadinene	3.2	-	1.5	-	1.8	1.7	1.7
	Total	14.1	8.3	16.9	9.7	41.3	28.1	55.1
				Oxygenated sesquiterpenes				
1507	Sesquicineole	-	-	-	-	-	19.9	-
1545	*E*-Nerolidol	-	-	-	-	-	-	-
1570	Spathulenol	1.8	5.1	2.7	15.5	12.8	2.5	6.5
1574	Caryophyllene oxide	0.3	5.2	-	10.0	-	-	0.7

1577	Globulol	-	-	-	22.0	6.6	1.3	3.6
1579	Viridiflorol	13.4	11.9	15.5	2.2			
1580	*Epi*-globulol	-	-	-	-	5.7	1.5	2.5
1595	5-*Epi*-7-*epi*-α-eudesmol	-	-	-	-	2.2	0.1	-
1601	Humulene epoxide II	-	0.5	-	0.5	-	-	0.1
1605	*Iso*-spathulenol	-	-	-	1.0	12.1	-	-
1607	1,10-Di-*epi*-cubenol	0.2	-	0.2	-	-	-	0.2
1614	Muurola-4,10(14)-dien-1-β-ol	-	-	-	-	-	-	-
1616	10-*Epi*-γ-eudesmol	-	-	0.2	-	1	-	0.4
1618	Caryophylla-4(12),8(13)-dien-5-α-ol	-	0.3		0.4	-	-	-
1620	1-*Epi*-cubenol	0.4	-	0.2	-	1.4	-	1
1622	Caryophylla-4(12),8(13)-dien-5-β-ol	-	1.1		1.5	-	-	-
1633	Cubenol	-	-	0.2	-	-	-	-
1637	*Tau*-cadinol	4.4	2.2	-	-	-	-	7.1
1638	Cubenol	0.4	-	-	-	-	0.7	-
1643	α-Muurolol	4.3	0.3	3.4	3.3	-	-	0.2
1649	α-Cadinol	-	1.5	-	1.2	1.6	1.6	0.9
1666	14-Hidroxi-9-*epi*-*E*-caryophyllene	-	-	-	-	0.6	-	0.2
1673	*Epi*-α-bisabolol	-	-	-	-	-	16.4	-
1681	α-Bisabolol	-	-	-	-	-	1.3	-
1684	Germacra-4(15),5,10(14)-trien-1-α-ol	-	-	-	-	-	0.2	-
2026	*E*,*E*-geranyl linalool	-	-	-	-	0.4	-	0.6
	Total	25.1	28.1	22.4	57.6	44.4	45.5	24.0
	Acids							
1780	Tetradecanoic acid	-	-	-	-	-	1.2	-
1974	Hexadecanoic acid	-	-	-	-	0.2	12.3	0.1
	Total	0	0	0	0	0.2	13.5	0.1

				Diterpenes				
2114	C20	-	2.5	-	0.3	-	-	-
2115	C20	-	0.3	-	1.0	-	-	-
	Total	0	2.8	0	1.3	0	0	0
				Triterpenes				
2994	Lanosterol	-	trace	-	0.8	-	-	-
3025	α-Amyrin	-	2.3	-	5.0	-	-	-
3052	β-Amyrin	-	trace	-	2.7	-	-	-
	Total	0	2.3	0	8.5	0	0	0
				Aliphatic				
969	6-metil-5-hepten-2-ona	-	1.6	-	-	-	-	-
1041	3-methyl-2-cyclohexen-1-one	-	3.2	-	-	-	-	-
	Total	0	4.8	0	0	0	0	0

[a] RRI: Relative Retention Index determined in reference to a homologous series of *n*-alkanes on a DB-5 column. HD: hydrodistillation.

Supercritical fluid extraction of C. calycireduplicatus* and *C. cuchillae-nigrae

Traditionally, essential oils are obtained through steam distillation and solvent extraction. However, alternative extraction methods, such as supercritical fluid extraction, have been used to isolate organic compounds from several natural products. Therefore, considering the principle of the technique, which uses variations in pressure and temperature to obtain different chemical groups, two *Croton* species were subjected to this technique to obtain different kinds of terpenes.

Croton calycireduplicatus and *C. cuchillae-nigrae* were subjected to supercritical CO_2 extraction with pressure variation. The yields of supercritical extracts of *C. calycireduplicatus* at pressures of 80, 150, 200, 250, and 300 bar were 0.43%, 0.42%, 0.29%, 0.53%, and 0.19%, respectively. The yields of supercritical extracts of *C. cuchillae-nigrae*, obtained at the same pressures, were quite similar: 0.29%, 0.38%, 0.32%, 0.49%, and 0.21%, respectively.

Figure 2 shows the chromatograms obtained by GC-MS of the essential oil from the leaves of *C. calycireduplicatus* extracted by the hydrodistillation (HD) and supercritical fluid (SFE) methods. Likewise, Figure 3 shows the chromatograms obtained for *C. cuchillae-nigrae* under the same conditions. The orange demarcated zone represents the region where monoterpenoids are observed, the green represents sesquiterpenoids, the yellow represents compounds from C16 to C26 and the red zone, C30. Basically, for both species, HD extracts only the essential oils, which are composed of monoterpenes and sesquiterpenes in both cases. Supercritical CO_2 extraction at 80 bar pressure yielded monoterpenes, sesquiterpenes, and higher molecular weight terpenes (starting at 65 min). At this pressure, sesquiterpenes are predominantly obtained, with very low quantity of monoterpenes, when compared with HD. As pressure increases, monoterpenoids are no longer detected and the sesquiterpenoid content decreases. This information can be seen in the decrease in their abundance value on the y-axis. From 150 bar it is possible to see the presence of α-amyrin and β-amyrin, until, applying 300 bar, these triterpenes are no longer observed. In the yellow region, it is possible, with the help of the Nist database, to verify, basically, the presence of long-chain hydrocarbons, such as alkanes, branched or not, with the presence or absence of functional groups, such as alcohols and aldehydes.

The fraction obtained by SFE from *C. calycireduplicatus* at 80 bar showed the presence of the following volatile components: α-tujene (0.05%), α-pinene (0.40%), β-pinene (5.41%), *p*-cymene (1.75%), limonene (0.24%), 1,8-cineole (0.41%), linalool (0.34%), *trans*-pinocarveol (0.67%), terpinen-4-ol (1.54%), α-terpineol (0.34%), β-caryophyllene (0.65%), aromadendrene (0.28%), α-humulene (0.09%), *allo*-aromadendrene (2.63%), α-muurolene (0.35%), spathulenol (5.13%), caryophyllene oxide (5.19%) and viridiflorol (11.93%).

The SFE-80 bar of *C. cuchillae-nigrae* presented the following volatile components: 1,8-cineole (0.29%), α-terpineol (0.59%), β-caryophyllene (0.21%), aromadendrene (3.28%), *allo*-aromadendrene (3.04%), α-muurolene (0.33%), γ-cadinene (2.15%), spathulenol (15.51%), viridiflorol (2.23%) and α-muurolol (3.33%).

These compounds were also present in the respective essential oil obtained by HD, but the concentrations were quite different. This result was not surprising since, by SFE, other non-volatile compounds are also obtained, causing the volatiles to be diluted.

In addition to several unidentified minor components, which were characterized as branched long-chain compounds (C26, C28, C30). Several C20 compounds were detected in minimal concentrations. However, it was not possible to identify them.

Triterpenoids, including α- and β-amyrin, have been reported for *Croton* species (Salatino et al., 2007). Extraction of α- and β-amyrin by SFE was already reported. Extractions were performed at pressures varying in the range 150–300 bar at fixed temperature of 60 °C. The best pressure for increasing α-/β-amyrin yield was 20 bar. (16,21). These data corroborate the results of the present study in which triterpenes were obtained at higher pressures.

Since diterpenes are compounds very frequently found in *Croton* species, there was an expectation of finding such compounds. However, in the species subjected to SFE, no peaks whose fragmentation indicated that they were diterpene compounds were identified in the chromatograms. Nevertheless, according to Salatino et al. (7), a possible trend in the chemical evolution of *Croton* is the loss of diterpenoids, with a shift towards mono- and sesquiterpenes, which are mainly present as volatile oil constituents. In fact, only a few species have been documented to contain both diterpenoids and volatile oils concurrently.

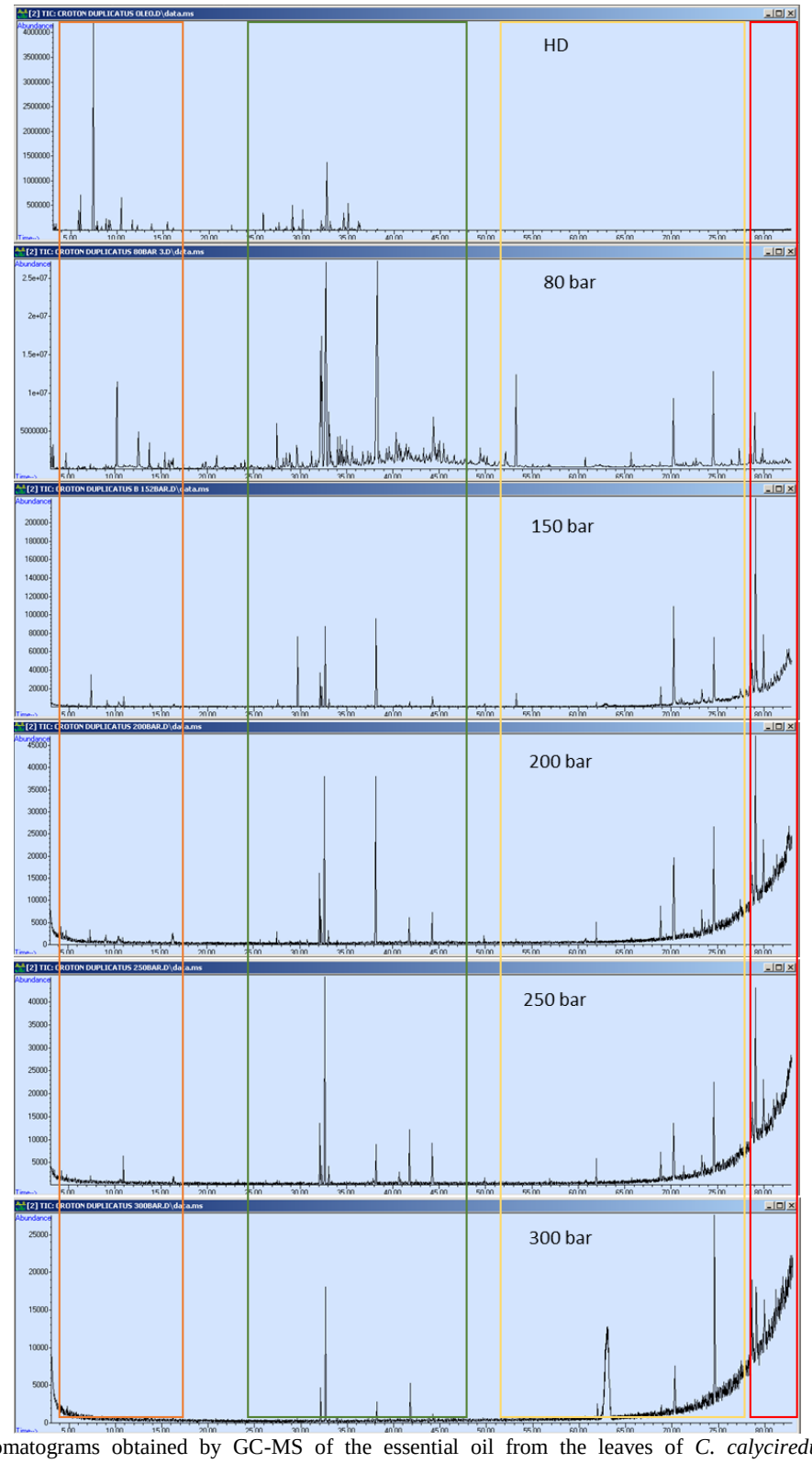


Figure 2. Chromatograms obtained by GC-MS of the essential oil from the leaves of *C. calycireduplicatus* extracted by the hydrodistillation (HD) and supercritical fluid (SC) methods in the pressure of 80, 150, 200, 250 and 300 bar. **O**range demarcated zone represents the region with monoterpenoids, green represents sesquiterpenoids, yellow represents compounds from C16 to C26 and the red zone, C30.

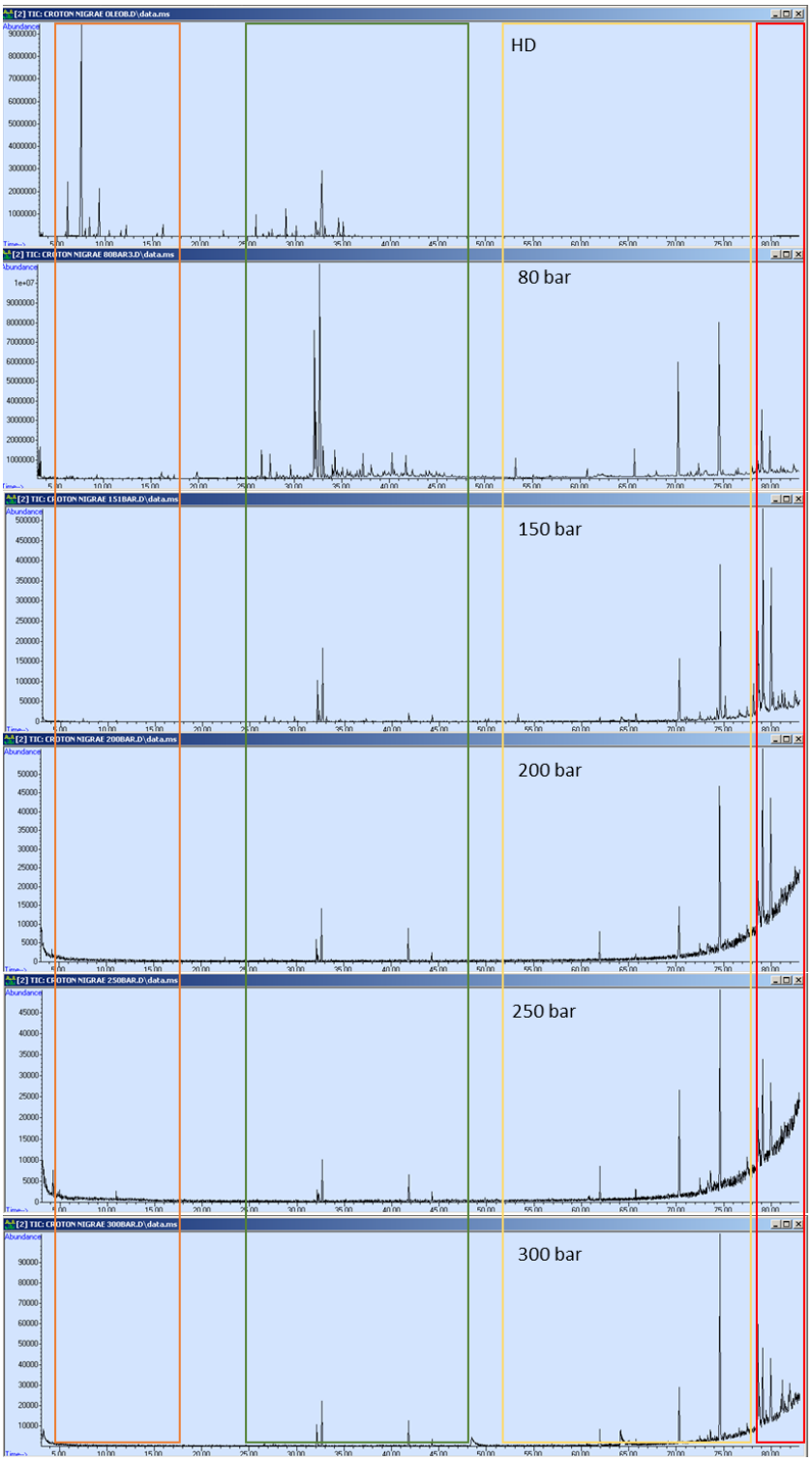


Figure 3. Chromatograms obtained by GC-MS of the essential oil from the leaves of *C. cuchillae-nigrae* extracted by the hydrodistillation (HD) and supercritical fluid (SC) methods in the pressure of 80, 150, 200, 250 and 300 bar. Orange demarcated zone represents the region with monoterpenoids, green represents sesquiterpenoids, yellow represents compounds from C16 to C26 and the red zone, C30.

Conclusions

This investigation into five *Croton* species revealed significant variations in essential oil yields (0.01% to 1%) and chemical compositions from their aerial parts. *Croton calycireduplicatus* and *C. cuchillae-nigrae* exhibited the highest yields (1%) and were rich in monoterpenes, particularly β-pinene. In contrast, *C. pycnocephalus* and *C. gnaphalii* essential oils were predominantly composed of sesquiterpenes, with bicyclogermacrene as the main component, while *C. hilarii* yielded the lowest amount and contained only sesquiterpenes.

Supercritical Fluid Extraction (SFE) applied to the high-yielding species (*C. calycireduplicatus* and *C. cuchillae-nigrae*) demonstrated pressure-dependent selectivity, extracting mono and sesquiterpenes at lower pressures and triterpenes (e.g., amyrins) and hydrocarbons at higher pressures, which aligns with existing literature on amyrin extraction by SFE. Despite these findings, SFE yields were generally low, suggesting non-optimal extraction conditions for the studied plants.

Notably, although diterpenes are frequently found in *Croton* species, none were identified in the SFE extracts. This absence supports the hypothesis of a chemical evolutionary trend in *Croton* involving the loss of diterpenoids and a shift towards mono- and sesquiterpenes, which are prevalent volatile oil constituents.

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Conflict of interest

The authors declare no conflict of interest.

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