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Fixed and volatile constituents of *Croton heliotropiifolius* Kunth from Bahia-Brazil

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Essential oils from *Croton heliotropiifolius* Kunth roots and stems, popularly known in Bahia (Brazil) as velame or cassutinga, were obtained by hydro distillation. Fixed oil from the roots of this species was obtained by maceration in hexane. The oils were analyzed by nuclear magnetic resonance (NMR) and gas chromatography coupled with mass spectrometry (GCMS). 46 compounds were identified in the stems and 35 substances were found in the roots. The principal components oils were camphor, β-pinene, and α-pinene in the stems, and camphor, borneol, valencene, and viridiflorol in the roots. The fixed oil contained C8 to C18 fatty acids. It is the first study that reports the presence of fixed oil from the roots of this genus.

Key words: *Croton heliotropiifolius*, Euphorbiaceae, essential oils composition, fixed oil composition, velame.

INTRODUCTION

Croton is distinguished by its morphological diversity and interintraspecific and it is the second largest genus of Euphorbiaceae, comprising around 1,200 species distributed in tropical and subtropical regions, particularly in America. Brazil is a country that congregates a large number of species, about 350 species (Lima and Pirani, 2008).

Several *Croton* species have been used in traditional medicine for different purposes (Randau et al., 2004; Vunda et al., 2012). The genus contains various substances with biological activities such as against cancer – diterpenoids (clerodane, furoclerodane and acyclic diterpenes), alkaloids (taspine), and essential oils (Salatino et al., 2007). Essential oils have also been evaluated for their larvicidal efficacy against *Aedes aegypti*, amoebicidal, antifungal, and antimicrobial activities (Salatino et al., 2007; Souza et al., 2006; Rodrigues et al., 2009; Motta et al., 2013). These oils present variable components depending on the climate and soil conditions in which the specimens are found (Maia and Ming 1998).

Amongst the species of *Croton* is found *Croton heliotropiifolius* Kunth (synonymy of *Croton rhamnifolius var. heliotropiifolius*), popularly known in Bahia (Brazil) as

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velame or cassutinga, is an aromatic plant, endemic species in the Northeast of Brazil, used in folk medicine as tea for gastrointestinal diseases and to alleviate fever (Randau et al., 2004). Depending on its location, *C. heliotropiifolius* shows great variation in size and shape of the leaves, fuzz color and length of inflorescences. It is a shrubby species that ranges from 0.7 to 2.5 m in height; it may have colorless latex or orange when oxidized. The branches are cylindrical and gray-green. Alternating leaves sub-opposite the apex of the branches and may be the entire or serrated margin. It can be distinguished from other species mainly by tripartite columella of fruit at the apex after dehiscence of the fruit (Silva et al, 2010).

Some previous studies presented variations in the composition of oils from *C. heliotropiifolius* stems due to the collection period and the region where the specimen was collected (Araújo-Neves and Camara, 2012; Souza et al. 2010). Araújo-Neves and Camara (2012) identified the volatile constituents of this species present in the stems collected in the region of Pernambuco, Brazil, identified guaiol (18.38%), β-elemene (17.28%) and valerianol (10.62%) as significant components of the oil. Souza et al. (2010) identified as major components of oil from stems as the α-pinene, β-pinene, camphor and germacrene D and found a significant seasonal variation in major constituents, especially the decrease in concentration in the dry season (winter).

Considering the above, this study aimed to analyze the chemical composition of volatile and fixed oils extracted from the stems and roots of *C. heliotropiifolius* Kunth and to check the alterations of the constituents according to region where the specimen was collected. Moreover, this is the first phytochemical investigation of constituents of fixed and volatile oils presents in the roots of *Croton*.

MATERIALS AND METHODS

Plant material

Roots and stems were collected in the Morro do Chapéu City located at 1011 m above sea level with highland tropical climate in the Chapada Diamantina, Bahia, Brazil. The *Croton heliotropiifolius* was identified and a voucher specimen is deposited in the Alexandre Leal Costa Herbarium of the Institute of Biology, at the Federal University of Bahia, Brazil under number 106168.

Oil isolation

Around 100 g of material was triturated and then subjected to hydro distillation for three hours in a modified Clevenger-type apparatus. The oil was collected from a condenser, dried over anhydrous sodium sulfate, and stored at 4°C till it was analyzed. The other portion of dried roots (784 g) of *C. heliotropiifolius* was macerated with 500 mL of *n*-hexane at room temperature. The oil was filtered and 6 mL were obtained. The fixed oil was purified by column chromatography in silica gel eluted with 20 mL of *n*-hexane and dichloromethane (8:2).

Chemical analysis of the oils

The oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS), the analyses were performed on a Shimadzu QP 2010 model mass spectrophotometer with ionization source of 70 eV and with a split/splitless injector, and a Shimadzu AOC-20i auto injector (Shimadzu, Kyoto, Japan). The volume of 1.0 µL of pure oil for each sample was injected at 220°C in a DBWAX column (polyethylene glycol) 30 m × 0.25 mm × 0.25 µm film thickness (Agilent J&W DB-WAX, Santa Clara, California). The analysis occurred with 1-minute sample time in the split mode, a column flow of 1.3 mL/min, a linear velocity of 41.4 cm/s, and scan between *m/z* 40 and 500. The oven temperature was initially set at 50°C. It was increased by 20°C/min until 240°C was reached, maintaining this temperature for 5 min. The temperature was then once again increased this time by 5°C/min until a temperature of 280°C was reached, maintaining this temperature for 30 min. Inlet pressure: 37.1 kPa. Carrier gas: He, linear velocity ($\bar{u}$): 32.4cm/sec. Injection mode: split (10:1). For identification of compounds, the peaks were compared with some standards, always consulting the libraries WILEY version nº. 7 and NIST version nºs. 12 and 62.

Identification of constituents of oils

The chromatograms are shown in Figure 1 and their compositions are presented in Tables 1 to 3. The constituent's fragmentation patterns in the mass spectra were compared with those from the libraries WILEY version nº 7 and NIST version nº 12 and 62.

RESULTS AND DISCUSSION

Essential oil composition

Sesquiterpenes were the major components in all the root and stem oils studied. These results suggest that regarding the distribution and accumulation of monoterpenes and sesquiterpenes of the root and stem oil from *C. heliotropiifolius*, the chemical composition of the oils is similar to that obtained by Araújo-Neves and Camara (2012) and Souza et al. (2010).

The fatty acids present in the fixed oil were oleic, stearic, pelargonic, and palmitic acids. This was first observed in the family of the latter substance. Prior to this research, fixed oil was only obtained from the stems of *C. cajucara* Benth. (Souza et al., 2006).

Chemotaxonomic analysis

The variability in the composition of oils shows a characteristic chemical profile of the genus *Croton*.

In the stems, the main components found were the monoterpenes camphor, β-pinene, and α-pinene respectively. The α-pinene is a terpene with anti-inflammatory, antifungal, and antioxidant activity and was seen in others species of *Croton*. β–pinene, which has antifungal efficacy, was found in a smaller number of species such as *C. argyrophylloides, C. zenhtneri*, and *C. nepetaefolius* (Morais et al., 2006).

Table 1. Major constituents in the essential oil stems (OS) from *C. heliotropiifolius*

Compound	RT	Area %	IRlit[15]	I.R exp
α-thujene	5.67	0.17	924	927
α-pinene	5.90	7.21	932	933
camphene	6.37	2.27	946	943
β-pinene	7.18	7.65	974	978
myrcene	7.46	0.35	988	991
p-cymene	8.68	0.52	1020	1025
limonene	8.85	0.59	1024	1030
eucalyptol	8.972	2.26	1033	1032
linalool oxide trans	10.37	0.20	1084	1086
linalool oxide cis	10.99	0.44	1067	1069
linalool	11.52	3.54	1095	1098
octanediol	11.71	0.29	1079	1073
pinocarveol (trans)	13.23	0.72	1135	1141
camphor	13.53	17.97	1141	1149
pinocarvone	14.11	0.17	1160	1164
borneol	14.55	4.50	1165	1173
myternol	14.81	0.18	1194	1191
terpineol	14.90	1.91	1130	1137
α-terpineol	15.53	3.24	1186	1195
tridecenyne	16.02	0.27	1319	1319
thymol	19.63	0.47	1289	1293
undecanone	19.78	0.27	1293	1294
geranyl acetate	23.45	0.65	1379	1380
β-elemene	23.90	0.18	1389	1398
caryophyllene	25.14	1.20	1417	1424
azulene	25.79	0.44	1491	1490
humulene	26.62	0.29	1452	1454
germacrene D	27.68	1.03	1484	1487
eremophilene	27.95	0.59	1486	1491
valencene	28.26	2.99	1496	1492
α-amorphene	28.41	0.98	1483	1482
d-cardinene	29.20	0.22	1522	1518
germacrene B	30.77	0.87	1559	1557
nerolidol	30.95	0.72	1561	1564
ledol	31.82	2.14	1590	1593
guaiol	32.28	0.69	1600	1614
rosifoliol	32.66	0.63	1600	1609
γ-eudesmol	32.93	2.05	1630	1632
intermedeol	33.06	0.78	1665	1647
humulene epoxide II	33.40	2.14	1608	1613
spathulenol	33.47	1.11	1577	1576
hinesol	33.78	2.37	1640	1645
α-cadinol	34.07	1.19	1652	1659
β-patchoulene	34.43	2.55	1432	1379
β-eudesmol	34.48	3.90	1649	1593
elemol	35.01	1.16	1548	1522

Camphor is an antiseptic found in the species *C. crassifolius* Geisel (Yang and Deng, 2009), *C. regelianus* Muell. Arg. (Torres et al., 2008), *C. cajucara* Benth (Souza et al., 2006) and *C. hieronymi* Griseb and the

Table 2. Major constituents in the essential oil roots (OR) from *C. heliotropiifolius*

Compound	RT	Area %	IRlit[15]	I.R exp
α-Pinene	5.90	3.92	933	933
Camphene	6.36	1.73	946	943
β-Pinene	7.18	2.73	974	978
Cymene	8.68	0.29	1020	1025
Limonene	8.85	0.37	1024	1030
Eucalyptol	8.97	3.08	1033	1032
Linalool	11.51	1.80	1095	1101
Camphor	13.51	15.21	1141	1149
Camphene hydrate	13.83	0.35	1145	1156
Borneol	14.57	12.05	1165	1173
Myrtenol	14.83	0.57	1191	1191
Terpinenol	14.90	1.38	1130	1137
α-Terpineol	15.53	2.58	1186	1195
thymol	19.63	0.32	1289	1293
Methyl eugenol	24.35	0.32	1403	1361
Cyperene	24.45	0.33	1403	1407
Caryophyllene (E)	25.14	0.67	1417	1424
Azulene	25.80	1.57	1298	1290
Eremophilene	27.96	2.70	1486	1491
Valencene	28.27	9.43	1496	1492
α-Guaiene	28.42	1.31	1436	1438
Elemol	30.38	0.79	1548	1546
Nerolidol	30.94	0.51	1561	1564
Spathulenol	31.82	0.36	1577	1576
Ledol	32.16	0.45	1602	1530
Guaiol	32.28	1.02	1600	1614
Rosifoliol	32.65	0.48	1600	1609
Humulene epoxide II	32.75	0.86	1608	1630
γ-Eudesmol	32.93	1.49	1630	1632
Intermedeol	33.06	1.42	1665	1668
Globulol	33.40	1.95	1590	1530
Hinesol	33.78	1.25	1640	1645
α-Muurolol	34.18	1.91	1644	1651
Viridiflorol	34.46	8.63	1592	1594
Hexadecanoic acid	48.04	0.53	1959	1987

Table 3. Major constituents in the fixed oil roots from *C. heliotropiifolius.*

Compound	RT	Area %	IRlit[15]	I.R exp
nonanoic acid (pelargonic)	25.72	7.2	1267	1371
methyl-hexadecanoate (methyl palmitate)	44.14	14.27	1927	1925
n-hexadecanoic acid (palmitic)	45.30	13.84	1959	1977
9-octadecenoic acid (oleic)	49.65	20.87	2077	2085
octadecanoic acid (stearic)	50.51	10.04	2141	2167

borneol in *C. hieronymi* Griseb (De Heluani et al., 2005) and *C. zambesicus* (Usman et al., 2009).

In the roots, the monoterpenes camphor (antiseptic)and borneol (gastroprotective), the sesquiterpenes valencene

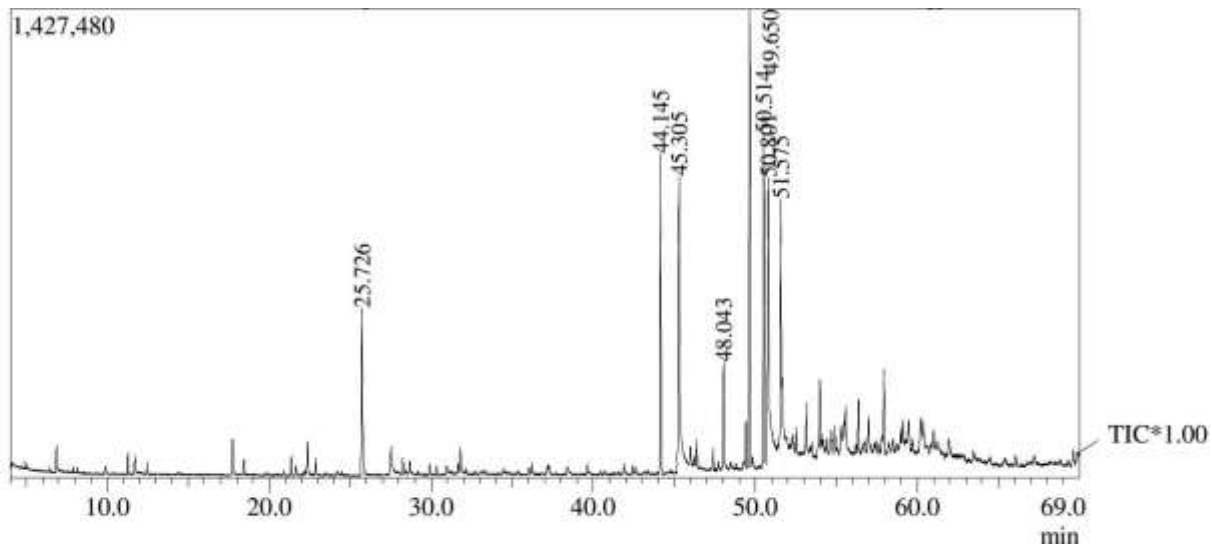

Figure 1. Cromatogram of fixed oil from roots from *C. heliotropiifolius.*

(flavor and mild oxidant) and viridiflorol (antiseptic, antibacterial, and anti-inflammatory) are the main constituents. Valencene was found in the leaves of *C. matourensis* and flowers of *C. micans* Swarts and viridiflorol has not been found in others species of *Croton* (Compagnone et al., 2010). Borneol is reported principally in barks of *Croton urucurana* (Simionatto et al., 2007).

Conclusion

This paper describes for the first time the chemical composition of the fixed oil present in C. *heliotropifolius*'s roots, as well as the volatile oils found in the roots and stems of this species with some differences from previous studies, which may be related to the region wherein the plant was collected and the stress level in the specimen used was submitted. Besides contributing to chemosystematics of the genus, this study has the perspectives to investigate the biological activities of the extracts; the phytochemical study of the polar extracts and other parts of the plant, as well as research for the future use of oil as cosmetics. It emphasizes the importance of the study of *C. heliotropifolius* found in the semi-arid of Bahia in order to contribute to the knowledge of the caatinga biome, unique in the world and has huge variety of plant species, but is threatened by human action. This knowledge can help promote their better preservation, creating jobs and reducing regional inequalities through sustainable exploitation of this and other plant species.

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Conflict of interest

Authors declare that there are no conflicts of interest.

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