FIXED AND VOLATILE CONSTITUENTS OF GENUS *CROTON* PLANTS: *C. ADENOCALYX* BAILL - EUPHORBIACEAE

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ABSTRACT

This work describes the phytochemical analysis of *Croton adenocalyx* Baill (Euphorbiaceae), a plant that is representative of the species from Ceará State (Brazil). The GC-MS analysis of the essential oil, obtained by hydrodistillation of the leaves from *C. adenocalyx,* allowed the identification of eleven volatile constituents; the main components were identified as α-pinene (32.63%); bicyclogermacrene (13.96%); *trans*-caryophyllene (10.23%); germacrene D (10.14%); β-pinene (10.11%) and β-elemene (8.31%). The chromatographic purification of the ethanolic extract from the trunk bark, allowed the isolation and identification of the 6-methoxy-7-hydroxycoumarin (**1**) and 3´,5-dihydroxy-3,4´,7-trimethoxyflavone (**2**).

Keywords: Euphorbiaceae, *Croton adenocalyx*, Essential oil, GC-MS, Flavonoid, Coumarin.

RESUMEN

Este trabajo describe el análisis fitoquímico de *Croton adenocalyx* Baill (Euphorbiaceae), una planta representativa de las especies del Estado de Ceará– Brasil. El análisis por CG-EM del aceite esencial, obtenido por hidrodestilación de las hojas de *C. adenocalyx* Baill, permitió la identificación de diez constituyentes volátiles. Los componentes mayoritarios fueron identificados como α-pineno (32.63%), biciclogermacreno (13.96%), *trans*-cariofileno (10.23%), germacreno D (10.14%), β-pineno (10.11%) y β-elemeno (8.31%). La purificación cromatográfica del extracto etanólico de la corteza del tronco, permitió aislar e identificar la coumarina 6-metoxi-7-hidroxicumarina (**1**) y el 3´,5-dihidroxi-3,4´,7-trimetoxiflavona (**2**).

Palabras clave: Euphorbiaceae, *Croton adenocalyx*, aceite esencial, GC-MS, Flavonoide, Coumarina.

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INTRODUCTION

Many Euphorbiaceae are well known in different parts of the world as toxic and/or medicinal (De Lima, 2000; 2009). The high diversity of the described effects is a reflection of the high chemical diversity of this plant group. *Croton* is a large genus of the Euphorbiaceae family, comprising around 1,300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of both hemispheres (Vieira, 1999; Branco and Pizzolatti, 2002; Fuentes *et al.*, 2004). Several of these plants are widely used in folk medicine as cicatrizing, anti-inflammatory, anti-cancer, against gastric problems, to treat hemorrhoids, and as agents to control uterine hemorrhages (D'Albuquerque *et al.*, 1973; De Lima, 2000). *Croton* spp produce clear to reddish or yellow latex which is rich in secondary metabolites with biological and/or pharmacological potential, mainly alkaloids, flavonoids, terpenes, terpenoids and ricin-type toxins (De Lima, 2000; Araújo-Júnior, 2005).

The traditional uses of *Croton* spp. have frequently been confirmed by pharmacological assays. Recently, Salatino *et al.* (2007) presented a review on the chemistry and pharmacological activities of crude extracts and pure metabolites from *Croton* spp. used in traditional medicine. The biological activities registered for *Croton* metabolites include anti-hypertensive, anti-cancer, anti-plasmodial, anti-inflammatory, antimalarial, antimicrobial, antispasmodic, antiulcer, antiviral, myorelaxant, and cytotoxic.

Several species of the genus are aromatic, indicating the presence of volatile oil constituents. The essential oil of *C. cajucara* Benth has been reported with anti-inflammatory and antinociceptive (Bighetti *et al.*, 1999); gastroprotective (Hiruma-Lima *et al.*, 2000; antileishmanial (Rosa *et al.*, 2003; Anthony *et al.*, 2005), antimicrobial (Mendonca-Filho *et al.*, 2005) and anti-gastric ulcer (Hiruma-Lima *et al.*, 1999) activities. Similarly, the essential oil from *C. nepetaefolius* has been reported to have antiparasitic and cardiovascular effects (Magalhães *et al.*, 1998; Lahlou *et al.,* 1999), as well as antinociceptive (Abdon *et al.*, 2002), cardiovascular (Lahlou *et al.*, 2000, Lahlou *at al.*, 1999), and intestinal myorelaxant and antispasmodic effects (Magalhães *et al.*, 1998). The chemical composition of some essential oils from *Croton* spp. that have been previously documented are listed in Table 1.

This paper describes the GC/MS study of the essential oil of *Croton adenocalyx*, a representative of the species from Ceará State (Brazil), and the isolation of two components of the ethanolic extract from the trunk bark.

EXPERIMENTAL

Plant material:

Botanical material of *C. adenocalyx* Baill was collected at Caucaia, in Brazil's Ceará state. Prof. Afrânio G. Fernandes from the Institute of Biology (UFC) identified the vegetable material and a voucher specimen was deposited at the Prisco Bezerra Herbarium, UFC, under the number 26350.

Gas chromatography/mass spectrometry:

GC-MS analysis was carried out on a Hewlett-Packard Model 5971 GC/MS using a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness); helium as the carrier gas, flow rate of 1.0 mL min^{-1} and with split ratio of 1:30. The injector temperature was programmed from 35 °C to 180 °C at 4 °C/min and then from 180 °C to 250 °C at 10 °C/min. Mass spectra were recorded from 40 – 450 m/z. The individual identification of components was based on comparison of their mass spectra with those of the Wiley 275.L MS library and those described by Adams (2007) and

their retention indices compared with those reported in the literature (Adams, 2007; Radulovic *et al.*, 2006; Rondón *et al.*, 2006). Spectra were considered coincident if the similarity index was higher than 95%. The results are presented in Table 2.

Column chromatography was performed on silica gel (Merck 60, 70-230 mesh). The IR spectra were recorded on a Perkin Elmer 1000 spectrometer. Melting points were determined on a Mettler melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker 500 spectrometer (11.7 Tesla, 500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts δ (in ppm) are given from internal TMS.

Extraction and Isolation:

Samples of fresh leaves (1.0 kg) of *C. adenocalyx* were subjected to hydro distillation for 2 hours in a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and, after filtration, kept refrigerated before being analysed (Costa et al., 2008). The dried and powdered stems (1.75 kg) of *C. adenocalyx* were extracted at room temperature with hexane (two extractions per 24h), solvent evaporation under vacuum yielded 14.15 g of hexane extract. The plant material was further extracted using ethanol to provide 55.60 g of extract. Part of this ethanolic extract (24.00 g) was fractionated on a silica gel column using hexane, chloroform, ethyl acetate and methanol, yielding 0.97, 3.43, 3.60 and 13.97g of extract respectively. The hexane fraction (0.97 g) was chromatographed on a silica gel column with a gradient of hexane in $CHCl_3$ yielding fifty fractions. The coumarin 1 (277 mg) was purified from fractions 25-41 and the flavonoid 2 (77 mg) was purified from fractions 19-21, after gel permeation rechromatography on Sephadex LH-20 using MeOH: CH_2Cl_2 (1:1). The structures of both metabolites were established by comparing their spectroscopic data with those reported in the literature.

RESULTS AND DISCUSSION

The GC/MS analysis of the essential oil from C. adenocalyx leaves showed a total of 24 components, and identified eleven of them (95.21%), with yields ranging from 0.12 to 0.14% (w/w), on a fresh weight basis. The main constituents were two monoterpenes [α-pinene (32.63%) and β-pinene (10.11%)] and four sesquiterpenes

Table 2. Chemical composition of the essential oil, obtained by hydrodistillation, from leaves of *Croton adenocalyx* Baill (Euphorbiaceae). *

Leaves of *C. adenocalyx* Baill	Retention Index	*Relative Area* (%)
α-pinene	943	32.63
β-pinene	978	10.11
α-terpinene	1015	2.10
δ-elemene	1329	1.19
β-bourbonene	1372	1.82
β-elemene	1389	8.31
trans-caryophyllene	1415	10.23
α-humulene	1451	2.68
germacrene D	1422	10.14
biciclogermacrene	1495	13.96
germacrene A	1498	2.04
		Total = 95.21%

*Spectra were considered coincident if the similarity index was higher than 95%. We also use Kovats indices estimated by a computer program based on the least square linear regression that uses the retention times of a few known compounds in the chromatogram and compatibles Kovats indices from literature (Alencar *et al.*, 1990)

[β-elemene (8.31%), trans-caryophillene (10.23%), germacrene D (10.14%) and bicyclogermacrene (13.96%)] Table 2.

Bracho and Crowley (1966) initially suggested that the co-occurrence of α/β-pinene might be a characteristic of the Croton genus. However, the results from the analyses of a larger number of Croton spp. show that β-caryophyllene and linalool appear to be equally frequent as major constituents in the essential oil of many Croton species (Table 1). Recently, Radulovic *et al.* (2006) studied the composition of the essential oil of four Croton species from Madagascar (*C. antanosiensis, C. decaryi, C. geayi, C. sakamaliensis*) and found that the oil composition varies greatly depending on the geographical location of the plant, and on the organ of the plant that the oil was extracted: with β-caryophyllene and/or α/β-pinene normally being the main constituents.

Our results (Table 2) differ significantly from the ones found for Craveiro *et al.* (1990) to *C. adenocalyx* specie (to see Table 1), specially for bicyclogermacrene (13.96%), germacrene D (10.14%), α-terpinene (2,1%) and gergermacrene A (2.04%) which also contribute significantly to the composition of the oil (Table 2). On the other hand both works showed α-pinene as the main constituent.

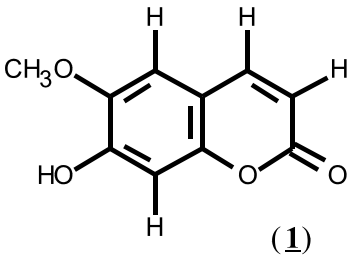
Some of the active components - monoterpenes and sesquiterpenes – found in the essential oil of *C. adenocalyx*, particularly α-humulene and β-caryophyllene, possess anti-inflammatory, analgesic, and antioxidant properties (Santos and Rao, 2000). α-pinene, one of the common monoterpenoids emitted from several aromatic plants, is known for its growth-inhibitory activity, and Martin *et al.* (1993) has investigated the anti-inflammatory activity in against rat hindpaw edema induced by carrageenin or by PGE_1. Germacrene D (Vagiona *et al.*, 2007) and biciclogermacrene (Silva *et al.*, 2007) are known for its antimicrobial activity. Similarly, α-terpinene has important properties such as acaricidal (Sanchez and Castanea, 2007), antioxidant (Takahashi *et al.*, 2003) and potent repellent activity (Choi *et al.*, 2002).

As can be seen in our results and other analyses of essential oil from the genus *Croton*, these plants have a tendency to biosynthesize high proportions of mono- and sesquiterpenes, independent of their natural habitats.

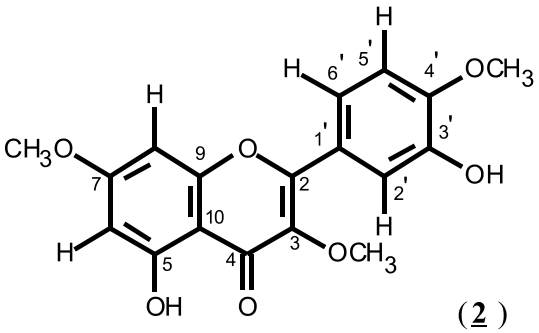
Fixed Constituents:

Purification of the hexane fraction, obtained from the ethanolic extract of the trunk bark of *C. adenocalyx* Baill, resulted in the isolation of coumarin (**1**) and flavonoid (**2**). The IR, ^{1}H and ^{13}C NMR spectroscopic data of **1** was in agreement with those reported for 6-methoxy-7-hydroxycoumarin (Mohamad Ali *et al.*, 2006; Silveira and Pessoa, 2005; Martins *et al.*, 2000), a coumarin isolated from *C. sonderianus* (Silveira and Pessoa, 2005) and reported to have cardiovascular properties (hypotensor), as well as antispasmodic and relaxant effects (Martins *et al.*, 2000). Coumarins are widely distributed in plants and are especially abundant in bark, leaves, and roots of *Umbelliferae* and *Rutaceae* (Masamoto *et al.*, 2004).

The flavonoid **2,** isolated for the first time in this specie, was obtained as yellowish needle-shaped crystals, m.p. 171.6-172.7 °C with dark purple absorption of the spot on TLC under long-wavelength UV light (366 nm). The UV spectra recorded with the classical shift reagents, clearly indicates that no free hydroxyl at C-7 and C-4'. Its IR spectrum showed absorption bands at 3400 cm^{-1} (OH); 1599, 1.498 and 1446 cm^{1} (C=C aromatic); 1651 (C=O conjugated carbonyl). It structure was suggested by ^{1}H and ^{13}C NMR spectral data together with the HMQC and HMBC experiments as 3´,5-dihydroxy-3,4´,7-trimethoxyflavone, whose spectral data are summarized in the Table 3. Comparison of these spectral data with literature values indicated that compound is identical to yanin, previously isolated from *Melicope hookeri* – Rutaceae



(**1**)



(**2**)

Table 3. ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) of 3´,5-dihydroxy-3,4´,7-trimethoxyflavone (2) from *C. adenocalyx* Baill:

	HMQC		HMBC	
C	**c**	**H**	**H ($^{2}J_{CH}$)**	**H ($^{3}J_{CH}$)**
4	178.9	----------	----------	----------
7	165.5	----------	6.42; 6.33	3.85
5	162.0	----------	6.33; 12.61	----------
9	156.8	----------	6.42	----------
2	155.7	----------	----------	7.70
4´	148.8	----------		3.97; 5.71; 7.67; and 6.95
3´	145.0	----------	5.71; 6.95	7.67
3	139.2	----------	----------	3.84
1´	123.6	----------	6.95	
10	106.1	----------	----------	6.33; 6.42; 12.61
CH				
6’	121.6	7.70 (dd, J = 2.2 and 8.8 Hz)	7.67	----------
2’	114.4	6.95 (d, J = 2.2 Hz)	----------	7.70; 5.71
5’	110.4	7.67 (d, J = 8.8 Hz)		
	----------	----------		
6	97.9	6.33 (d, J = 2.2 Hz)	----------	6.42; 12.61
8	92.2	6.42 (d, J = 2.2 Hz)	----------	6.33
CH_3				
OCH_3-3	60.2	3.84 (s)	----------	----------
OCH_3-4’	56.1	3.97 (s)	----------	----------
OCH_3-7	55.8	3.85 (s)	----------	----------
OH				
OH-3’	--------	5.71 (s)	----------	----------
OH-5	--------	12.61 (s)	----------	----------

(Mohamad Ali *et al.*, 2006) and from *Distemonanthus benthamianus* (Malan and Roux, 1979).

Finally, the ^{1}H - ^{1}H NOESY and HMBC experiment were important determination of the substituent group position. It showed correlations between the methoxyl group protons at δ 3.97 (in C-4’) and the proton at δ 7.67 (1H, d, J=8.8 Hz, H-5’), and between the methoxyl group protons at δ 3.85 (C-7) and the protons at δ 6.42 (1H, d, J=2.2 Hz, H-8) and δ 6.33 (1H, d, J=2.2 Hz, H-6). This last correlation confirmed the methoxyl group at C-7.

The flavonoid commonly known as ayanin has been isolated previously from other plant sources, including *C. schiedeanus* Schlecht (Guerrero *et al.*, 2002a; Gue-

rrero, *et al.*, 2002b; Puebla, *et al.*, 2005), *C. glabellus* (Garcia *et al.*, 1986), *Combretum quadrangulare* (Combretaceae) (Castedlen and Hall, 1989), *Psiadia trinervia* (Asteraceae) (Wang *et al.*, 1989), and which has been reported to have antimicrobial activity (Wang *et al.*, 1989), as well as vasorelaxant and protective cardiovascular effects (Guerrero *et al.*, 2002a). Some structural types of flavonoids have reportedly been obtained from *Croton* (D'Albuquerque *et al.*, 1973; Novoa *et al.*, 1985; Garcia *et al.*, 1986; Capasso *et al.*, 2000), and some are reported to be responsible for hypotensive (*C. glabellus*; Novoa *et al.*, 1985) and vasorelaxant effects (*C. schiedeanus*; Carrón *et al.*, 2010; Guerrero *et al.*, 2002b).

This study has been important from a pharmacological point of view, because the isolated components and essential oil has demonstrated beneficial cardiovascular effects (Salatino *et al.*, 2007; Siqueira *et al.*, 2005; Hiruma-Lima *et al.*, 2000; Lahlou *et al.*, 1999), which may explain or contribute to the use of crude extracts from Croton spp. utilized in traditional medicine (Salatino *et al.*, 2007). Furthermore, no phytochemical study of other parts of this plant has been described, and the chemical composition of the essential oil presented differs from previous reports.

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