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Flavonols and sesquiterpenoids from outer bark and leaves of *Croton polycarpus* Benth. (Euphorbiaceae)

[Flavonoles y sesquiterpenoides de la corteza y hojas de *Croton polycarpus* Benth. (Euphorbiaceae)]

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Abstract: Some known flavonols from the outer bark polar fraction of the native species *Croton polycarpus* Benth. (Euphorbiaceae) were isolated and quercetin, quercitrin and rutin were identified as the most abundant constituents. From this sample, a clean TLC non polar sub-fraction (A-1) obtained by CC, afforded several isomeric compounds, which were characterized by GC-MS/EI and its results conduct to a group of eighteen sesquiterpenoids with a unique Rf value in TLC; among them, one sesquiterpene hydrocarbon with molecular weight (MW) of 200 g/mol ($C_{15}H_{20}$), two with MW 202 g/mol,($C_{15}H_{22}$), thirteen with a MW of 204 g/mol ($C_{15}H_{24}$) and two sesquiterpenols with MW 220 g/mol ($C_{15}H_{24}O$) were determined. From its fresh leaves essential oil obtained by two methods, hydro-distillation and supercritical CO_2 extraction, other terpenoids were identified, where the first one process was more efficient (1.5% DM yield) than the last one, and it was selected for a pilot study. This is the first report about chemical composition of the native species *C. polycarpus*, and the analysis of sesquiterpene isomerism is very important to recognize its structural diversity and similar chemical behavior.

Keywords: Flavonoids, sesquiterpenoids, isomerism, GC-MS, *Croton polycarpus*, Euphorbiaceae.

Resumen: De la fracción polar de la corteza de la especie nativa *Croton polycarpus*, (Euphorbiaceae) se separaron algunos flavonoles y quercetina, quercitrina y rutina se identificaron como los constituyentes más abundantes. Desde una fracción apolar de esta muestra, se obtuvo por CC una fracción limpia en CCD (C-2), cuyo fraccionamiento por análisis por CGAR-EM/IE permitió determinar un grupo de 18 sesquiterpenoides mayoritarios los cuales presentan un valor único de Rf; entre estos se detectó un hidrocarburo de PM 200 ($C_{15}H_{20}$), dos derivados de PM 202 ($C_{15}H_{22}$), trece son hidrocarburos isómeros con PM 204 g/mol ($C_{15}H_{24}$) y dos terpenoles de PM 220 uma ($C_{15}H_{24}O$). De igual forma, de las hojas frescas se extrajo el aceite esencial, por dos métodos: hidrodestilación y extracción con CO_2 supercrítico (SFE), y el primero de ellos fue el más eficiente (1,5% rendimiento en peso seco) por lo cual se seleccionó para el desarrollo de experimentos a escala de planta piloto. Este informe es la primera contribución al conocimiento de la composición química y la bioactividad de las hojas y corteza de la especie arbórea nativa *Croton polycarpus* (Euphorbiaceae) y el análisis de mezclas de sesquiterpenos isómericos es muy importante para reconocer la diversidad estructural y su similitud en polaridad y comportamiento químico.

Palabras clave: Flavonoles, sesquiterpenoides, GC-MS/EI, *Croton polycarpus*, Euphorbiaceae.

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INTRODUCTION

The Euphorbiaceae family is worldwide distributed and contains trees, shrubbery and herbs. In Colombia, it is represented by 78 genera, 390 species, 12 subspecies and 9 varieties, which has been increased along the last years with the introduction of new species belonging to genera *Mabea, Croizatia* and *Pausandra*. Within the most representative genera, it is *Euphorbia* with 43 species (spp.), *Croton* (80 spp.), *Phyllantus* (36 spp.), *Acalypha* (25 spp.), *Alchornea* (19 spp.), *Mabea* (18 spp.) and others that present less than 11 species (Murillo, 1999; Murillo, 2004).

Chemical composition and biological activity in Euphorbiaceae have gotten some advances related mainly with *Euphorbia*, *Alchornea* and *Croton* taxa. Regarding *Croton* genus, there are extensive reports about bioactivity, chemical composition, their biological properties and bioactive constituents; in this respect, it is worth mentioning several studies about the product known as "Sangre de Drago" which is a red resin with anti-cancer, anti-inflammatory, anti-oxidant and bactericidal activities, and it is obtained from numerous *Croton* species widely distributed in South America (Ramírez, 2003). This genus offers a great amount and variety of constituents like phorbol esters, alkaloids, di and triterpenoids as clerodanes, euphol derivatives, and flavonoids and their glycosides with notable medicinal applications, as summary in Table 1.

Table 1
Chemical composition and biological activity review of *Croton* genus.

Species	Place	Part	Compound isolated	Bioactivity	Reference
C. brasiliensis	Alagoas, Brasil	Stem and leaves	Dit. as crotobrasilin A and B	Not known reports.	Palmeira Jr. *et al.*, 2005
			Flav. as casticin, penduletin & crisospenol-D		
C bogotanus	Bogotá, Colombia	Leaves	Dit. as cassipourol		Vegas *et al.*, 2011
C. cajucara	Jacundá, Brasil	Bark	Cler. as *t*-cajucarine B. sacacarine. *t*-crotonine, cajucarinolide.	Antimicrobial, antiviral.	Aparecida *et al.*, 2002
			Trit. as acetilaleuritolic acid.		
		Leaves	Flav. as methylkaempferol.	Diabetes. cholesterolemy	Campos *et al.*,2002
			Ster. as β-sitosterol & stigmasterol.		
		Bark and stem	Cler. as *trans*-crotonine		Perazzo *et al.*, 2007
	Benfica, Brasil	Bark	Dit. as dehydrocrotonine	Antiulcer.	Silva *el al.*,2003
C. californicus	Arizona, USA	Bark	Dit. as methyl-barbascoate.	Tumor promoter.	Chavez *et al.*,1982
			Éster 12-deoxiphorbol.		
C. campestris	Sao Paulo, Brasil	Bark and roots	Cler. as velamone & velamolone.	hepatic diseases & syphilis	Babili *et al.*,1998
				Leishmaniasis	Babili *et al.*, 2006
C. caudatus	Yunnan, China	Stem	Sesq. as crocaudatol & oplopanone	Anticancer	Wang & Zou, 2008
			Flav. as Crotoncaudatin, tangeretin, nobiletin & sinensetin	Antimalaric and fever.	Zou *et al.*, 2010
C. celtidifolius	Santa Catarina, Brasil	Bark	Proantocianidin 63SF	Pain and wounds.	DalBó *et al.*, 2006
			galocatequin & catequin	Leukemia	Nardi *et al.*,2003
	Orleans City, Brasil			Antiinflammatory	Carvalho *et al.*, 2008
			Ster. as β-sitosterol.		
C. cuneatus	Barinas, Venezuela	Bark and stem	Terp. as α-11 eudesmen, julocrotonine & julocrotol	Analgesic and antiinflammatory	Suárez *et al.*, 2006
C. hovarum	Ankazobe, Madagascar	Bark	Trit. as β-amirine	Toxic compounds.	Krebs & Ramiarantsoa,1996
			Trit. as 4-hydroxyhigrinic acid		

C. kongensis	Yunnan-Tibet, China	Leaves	Dit. as kongensin & rabdoumbrosain	Antimalaric	Yang *et al.,* 2009
			4-hidroxibenzoic acid		
C. lechleri	Cerro de Pasco, Perú	Latex	Lign. as 3'4-O-dimetilcedrusin.	Antiinflammatory antiviral and antitumor.	Coussio *et al.*,1997
			catequin, epicatequin & galocatequin.		
			Proantocianidin SP-303.		
			taspin & apomorfin	Antioxidant and anticancer	Lopes e Lopes *et al.*, 2004
C. malambo	Santa Bárbara del Zulia, Venezuela	Bark	β-pinene, α-bergamotene, β-metil-isoeugenol, linalool	Analgesic and antiinflammatory	Suárez *et al.*,2003
	Puerto Colombia, Atlántico			Citotóxic and antiinflammatory	Jaramillo *et al.*, 2007
C: megalocarpus	Arusha, Tanzania	Essential oil	Fatty acids C16 and C18	Biodiesel.	Kafuku *et al*., 2010
C. membranaceus	Krobo-Gyakiti, Ghana	Roots	Alk as julocrotin	Not known reports	Aboagye *et al.*,2000
C. micradenus	Guantánamo Cuba	Stem and leaves	Alk as sinoacutin, cobotrin & soboldin	Antimalaric	Payo *et al.*,2001
C. oblongifolius	Petchaboon, Tailandia	Bark	Cemb. as crotocembraneic acid.	Dyspepsia treatment.	Roengsumran *et al.*, 1998
C. pullei	Pará, Brasil	Stem and bark	Alk as julocrotin, crotonimide A and B	Not known reports	Bárbosa *et al.*, 2007
C. schiedeanus	Tocaima, Colombia	Stem and leaves	Phenbut. as acetoxi & diacetoxirododendrol		Puebla *et al.*, 2005
C. sonderianus	Ceara, Brasil	Roots	Cler. as sonderianin	Antifungal, antibacterial.	McChesney *et al.*, 1991
C. sublyratus	Bangkok, Tailandia	Leaves	geranylgeraniol. & plaunotol.	Antibacterial against *H. pylori*.	Tansakul & De Eknamkul, 1998
C. tonkinensis	Vietnam	Leaves	Flav. as vitexine, isovitexine & tiliroside	Antioxidant, antitumor and antiinflammatory.	Giang *et al.*,2004
C. zambesicus	Yaounde, Camerún	Bark	Dit. as crotonadiol.& crotocorylifurane.	Laxative.	Ngadjuli & FoIefoc.,1999
			Trit. as lupeol. betusinol.		
			Ster. as 3β-glucopiranosil-sitosterol		
	Benin, África	Leaves	Dit. as *ent*-18-hidroxitraquilobane	Hypertension	Martinsen *et al.*, 2010
C. zehntneri	Pernambuco, Brasil	Roots	crototropone	Antioxidant & antiinflammatory.	Bracher *et al.*, 2008
	Ceará, Brasil	Leaves	Sesq. as *trans*-anetol & *trans*-cariophyllene	Sedative.	Rodrigues *et al.*, 2009

Dit: Diterpenoid; **Trit**: Triterpenoid; **Sesq**: Sesquiterpenoid; **Alk:** Alkaloid; **Ster**: Steroids; **Flav**: Flavonoids; **Cemb**: Cembranoids; **Terp**: Terpenoid; **Lign**: Lignane; **Phenbut**: Phenilbutanoid; **Cler:** Clerodane.

In Colombia, previous works include a report of two constituents of non-polar fractions from *Alchornea glandulosa*, where the presence of 3-hidroxifriedelin and 3-oxo-friedelin were determined, whose bioactivity in Brine Shrimp Test (BST) was EC_{50}= 31 μg/mL for the first compound and EC_{50}= 43 μg/mL for the last one (Tello-Camacho, 2005). Also for this

plant the biological activity of phenolic compounds isolated, was assessed (Urrea-Bulla *et al*., 2004).

From genus *Croton* some terpenoids have been obtained like linalool, germacrene D, *trans*-β-caryophyllene, and others from volatile fractions of *C. malambo* (Jaramillo *et al*., 2010) where eugenol and methyl-eugenol were the main constituents with potential cytotoxic and repellent activities (Jaramillo *et al.,* 2007; Muñoz-Acevedo *et al*., 2014; Mendoza-Meza *et al*., 2014). Likewise, it was found *trans*-calamenene, dihydrocurcumene and β-caryophyllene as more abundant constituents of essential oil (EO) of *C. trinitatis* (Jaramillo-Colorado *et al*., 2016) and cassipourol, which is a diterpenic alcohol with potential larvicidal activity, isolated from a non-polar fraction of *C. funckianus* senescent leaves. From the polar fraction of *C. funckianus*, some phenolic compounds were isolated and they were elucidated as quercetin and quercitrin, were the first one gave a promissory larvicidal activity [CE_{50} = 47 μg/mL] against mosquito larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae), and the last one was characterized as a flavonol glycoside without relevant biological activity (Vegas-Mendoza, 2010; Vegas *et al.,* 2011).

At the best of our knowledge, there are not previous reports about *Croton polycarpus* Benth. [Basiosym. *Oxidectes polycarpa* (Benth.) Kuntze] chemical composition or bioactivity, so this is the first contribution to its chemical analysis. The main goal of this research was to study the compounds of native species *C. polycarpus* grown on the Bogotá highlands. From its outer bark and fresh leaves by chromatographic methods as TLC, HRGC-MS/EI the essential oil (EO) chemical composition was analyzed; the EO was obtained by two methods: hydro-distillation and CO_2 supercritical extraction, with subsequent application of analytical chromatographic methods in order to select the best for larger scale experiments.

MATERIALS AND METHODS

Plant material

Bark trunks and fresh leaves of *C. polycarpus* were collected in August 2010 at the Universidad Nacional de Colombia campus located in Bogotá (UNC-SB), at 2600 AMSL, where a voucher specimen under the code COL 520458 is deposited at the Herbario Nacional Colombiano (HNC) in the Instituto de Ciencias Naturales (ICN) – UNC- Bogotá.

Extraction and Isolation

A *C. polycarpus* sample was separated manually in outer bark, wood and senescent leaves and then were dried and powered for subsequent solvent extraction. Firstly, a bark sample (720 g) was extracted with EtOH (96%) at RT by percolation, changing the solvent thoroughly. Filtered extract (F-1) was concentrated at reduced pressure (RP) at maximum 40°C in a rotatory evaporator (Heidolph WB2000). A portion of the extract (10 g) was fractionated by L-L partition between water and petroleum ether (F-2), $CHCl_3$ (F-3), AcOEt (F-4) *n*-BuOH (F-5) and aqueous residue (F-6) were obtained. After application of general BST with *Artemia salina nauplii* and specific larvicidal activity bioassay (LAB) with mosquito *Culex quinquefasciatus* larvae, a progressive chromatographic analysis by TLC was developed (McLaughlin *et al*., 1998).

According TLC results, fraction C-2 (1.12 g) was fractionated in a silica gel (70-230 mesh) column (PE/AcOEt 100:0→0:100 and then AcOEt/MeOH 90:10→10:90); 25 fractions eluted with PE/AcOEt 95:5 (96 mg) were collected, analyzed by TLC in hex/tol 50:50 and an unique spot (R_f = 60) was observed with acid reagent H_2SO_4:H_2CO:W(2:1.7) and heating at 120° C (H^+/Δ); a sample of 1 μL solution was subjected to CG-MS/EI with splitless injection method and lineal velocity of 37 cm/sec. The oven heating ramp program was 50° C (2 minutes), increasing until 200° C (4° C/min) and then up to 250° C (10° C/min).

Fresh leaves sample (500 g) was subjected to hydrodistillation at ATM (3 L/H_2O, 3 h) in a Clevenger-type apparatus, where the volatile oil was isolated by L-L partition with Et_2O and then dried over anhydrous Na_2SO_4 (yield 1.5%). After filtration, it was kept in amber-colored bottle at RT. Other leaves sample (25 g) was submitted for supercritical CO_2 extraction (1400 psi, 43° C, 0.3 g/cm^3 gas density) for 60 minutes (yield 0.1%). According these results a sample of EO obtained by HD was selected for CG-MS/EI with a same heating ramp program previously proposed.

Flavonols and their glycosides were isolated from the raw fraction in AcOEt (1.1 g), by successive CC (silica gel 70-230 mesh) with mixtures of toluene/AcOEt (100:0→0:100) and AcOEt/MeOH (100:0→0:100), where it was collected F-7 (10 mg), F-8 (13 mg) and F-9 (15 mg) fractions. The ^{1}H NMR (400 MHz) spectra afforded enough information to characterize the compounds as the known flavonol

quercetin and their glycosylated derivatives quercitrin (quercetin-3-O-α-L-rhamnoside) and rutin (quercetin-3-rutinoside) by comparison with previously reported values (Fathiazad *et al*., 2006).

General chromatographic conditions

Analysis by HRGC-MS for C-2 sample was performed on a Shimadzu QP2010 coupled with MS-EI detector QP-2010 S (70 eV) and an ion source temperature of 230°C, using a column packed with 5% phenil-95% dimethylpolysiloxane S-5 (30 m × 0.25 mm × 0.25 μm). HRGC-MS for essential oils was analyzed on a HP6890 Series II with HP-5973N MS detector (e.i. 70 eV) at 230° C, equipped with a fused silica capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm). In the same conditions, a mixture of linear hydrocarbons from C-8 until C-20 was run. The identification of compounds was made with application of two main criteria: the chromatographic one comparing data with retention indexes (KI) calculated based on the Kovats system related with the reference lineal paraffin's (C_8 to C_{20}) mixture, as well as with the spectroscopic criteria based on the grade of coincidence of the mass spectra registered with the reported in NIST08 and Wiley Registry 8e GC-MS libraries. ^{1}H NMR and ^{13}C spectra were registered in a Bruker MX-400 at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using MeOHd_4 and $Me_2CO d_6$ as dissolvent and TMS as internal standard.

RESULTS AND DISCUSSION

From BST results of EP-outer bark and AcOEt senescent leaves extracts obtained from *C. polycarpus,* under the protocol proposed by McLaughlin *et al*. (1998) it was found that these present promised bioactivity in outer bark, whose values are 58 μg/mL and 198 μg/mL (Table 2). Otherwise, senescent leaves present better bioactivity for its AcOiPr, *n*-BuOH and PE extracts with specific larvicidal activity bioassay (LAB) using *Culex quinquefasciatus* larvae. Based on these reports, the study of its chemical composition was conducted and the results agree with data coming from other *Croton* species (Vegas *et al*., 2014).

Table 2
BST and LAB results as EC_{50} for *C. polycarpus* of outer bark and senescent leaves extracts

Extracts	BST Bark (μg/mL) EC_{50}	LAB Senescent leaves (μg/mL) EC_{50}
F-1	315	105
F-2	198	549
F-3	229	>1000
F-4	149	---
F-5	---	494
F-6	---	>1000
F-7 (AcOiPr)	---	451
Caffeine	42	450

Fractionation of constituents of outer bark F-2 extract were made over a silica gel column (70-230 mesh) and led to C-2 clean fraction, whose BST EC_{50} value was 58 μg/mL, and shows a unique spot with R_f =60. Its chemical composition was studied by HRGC-MS, where more abundant compounds are a set of isomeric sesquiterpenoids according the profile observed in Figure 1.

Figure 1 records the chromatographic profile of sample C-2 coming from HRGC analysis joint with its respective enlargement in the range of 20 to 35 minutes of elution. The chromatogram allows to differentiate the Retention Time (R_T) of at least eighteen compounds, most of them with MW varying between 200 and 220 uma corresponding to sesquiterpenoid derivatives like sixteen hydrocarbons and two sesquiterpenols that by application of the chromatographic and spectroscopic criteria have conducted to detection of their identities complemented by fragmentography analysis. Sesquiterpene hydrocarbons with two or more rings and exocyclic double bonds exhibit spectra consistent with isomerization to cyclohexene derivatives that can suffer other rearrangements and afford aromatic fragments as seen in Figure 3 (Mc Lafferty & Turecëk, 1993). The mass spectra analysis conducted

to the identification of following known terpenoids: α-copaene [**1**], β-caryophyllene [**2**], aromadendrene [**3**], δ-cadinene [**4**], α-calacorene [**5**], δ-cadinol [**6**], 9,10-dehydro-isolongifolene [**7**] and (+)-cuparene [**8**] as most abundant constituents (Figure 2).

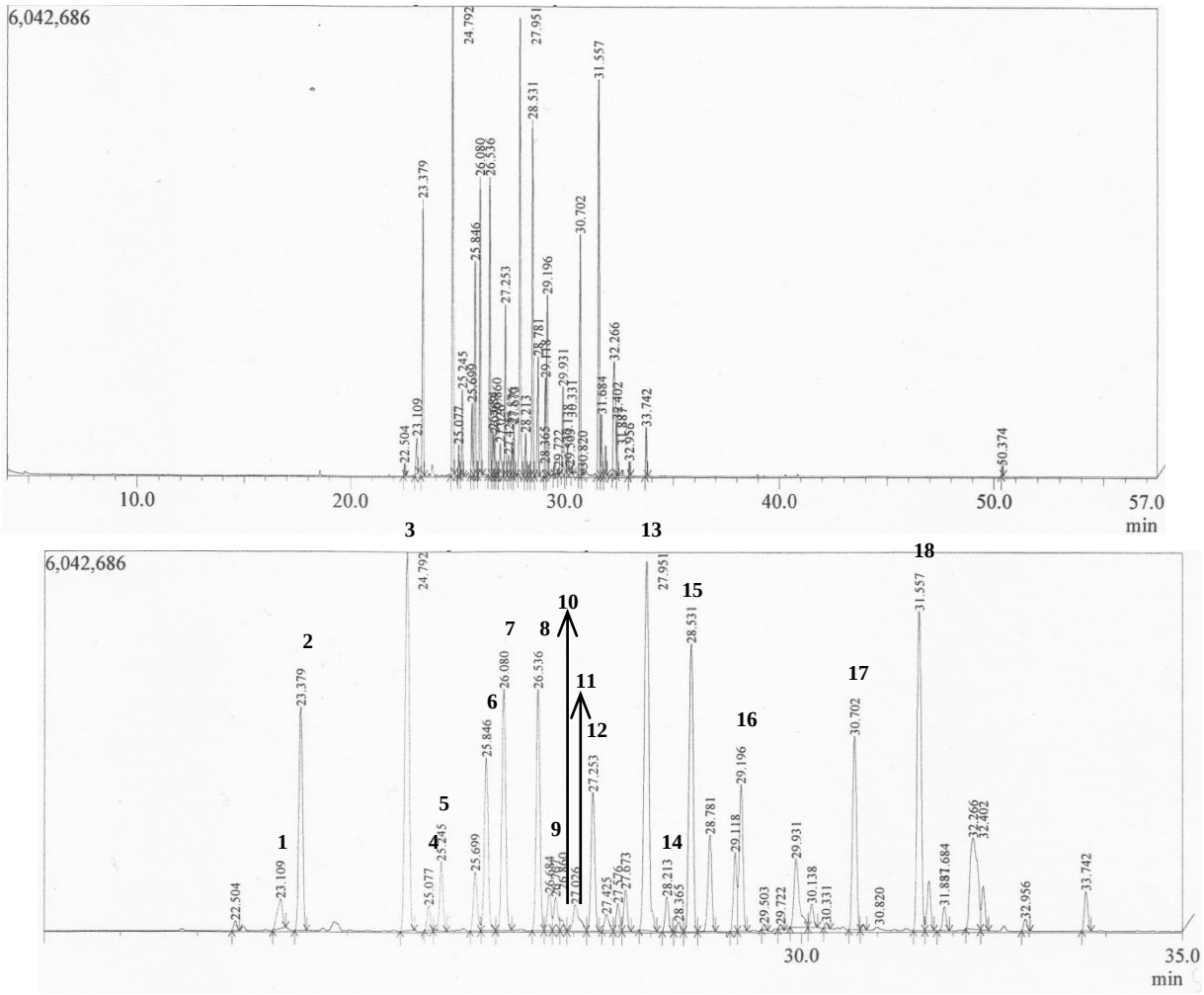


Figure 1
Total and enlargement chromatograms of C-2 fraction

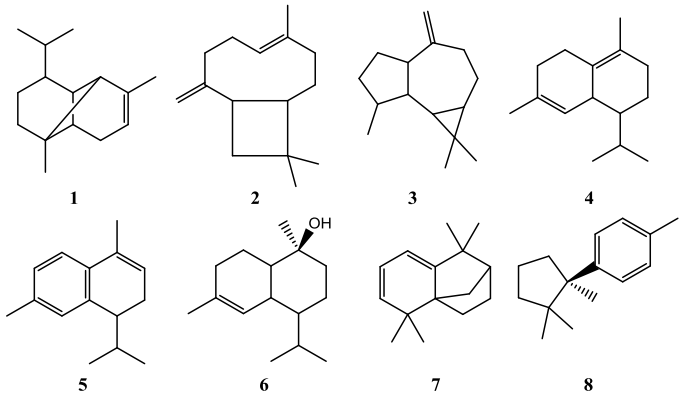
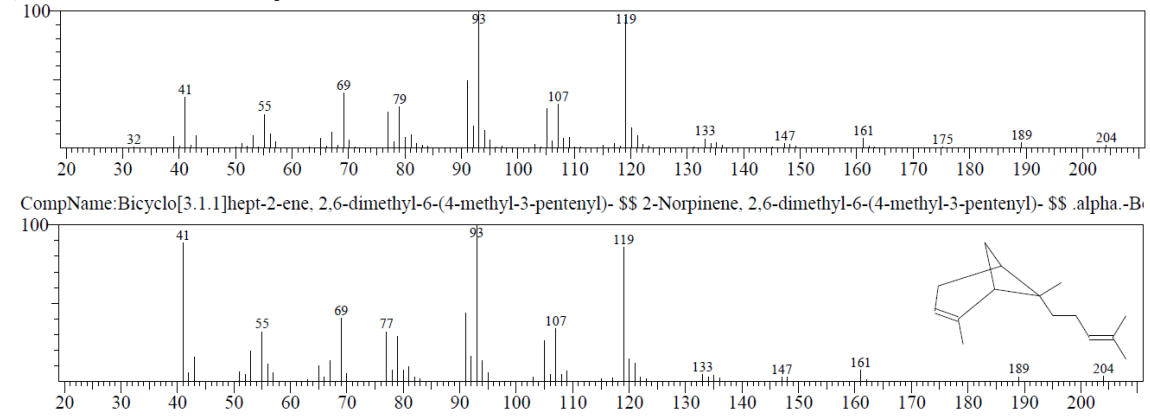
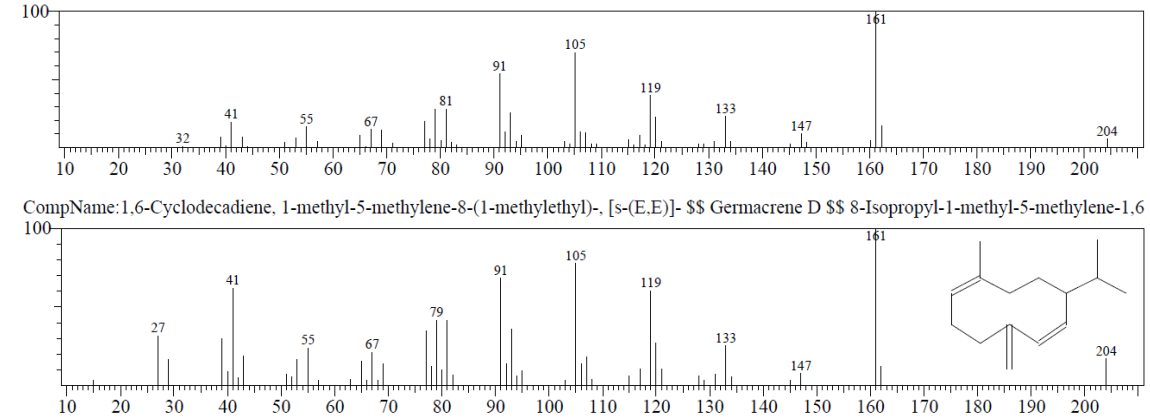


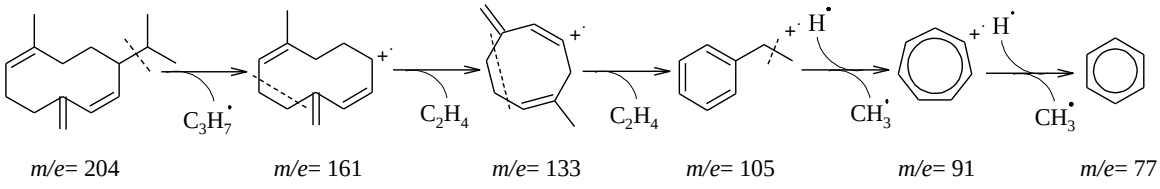
Figure 2
Structures of main compounds from outer bark C-2 fraction.

Table 3 shows the list of compounds identified by spectroscopic analysis of C-2-fraction which is mainly formed by thirteen isomeric sesquiterpenoid hydrocarbons of MW of 204 g/mol, most of them as decaline derivatives. On the other hand, there were found two other hydrocarbons of 202 g/mol known as (-)-calamenene and (+)-cuparene, two sesquiterpenols with 222 g/mol named δ-cadinol and germacren-D-4-ol and finally α-calacorene with a MW of 200 g/mol. Application of chromatographic and spectroscopic criteria, to each component, for example, to germacrene D [**4**] and α-bergamotene [**5**] show that both retention index with values of 1474 and 1476 are similar and present differences with literature reports based on different experimental conditions although their mass spectrum present a molecular ion of [M^+] = 204 uma; however, by comparative analysis the first one presents a base peak of *m/e* = 161 uma, while the last one has it at *m/e* = 93 uma, this led to confirm their structures.



[**4**]





[**5**]

Figure 3
MS spectra of [4] and [5] compounds with respective fragmentography for germacrene D

Table 3
Chemical composition and Area% from *C. polycarpus* C-2 fraction

Peak	I_R lit.	I_R exp.	Compound name	Formula	MW	Area%	Base Peak MS	Id. Criteria	I_R theor. Ref.
1	1364	1447	(+)-cicloisosativene	$C_{15}H_{24}$	204	1.05	105	MS	[a]
2	1376	1451	α-copaene	$C_{15}H_{24}$	204	5.79	105	MS	[a]
3	1418	1470	β-caryophyllene	$C_{15}H_{24}$	204	10.79	93	MS	[a]
4	1480	1474	germacrene-D	$C_{15}H_{24}$	204	0.56	161	MS	[a]
5	1436	1476	α-bergamotene	$C_{15}H_{24}$	204	1.63	93	MS	[a]
6	1454	1484	α-humulene	$C_{15}H_{24}$	204	4.56	93	MS	[a]
7	--	1487	unknown 1	$C_{15}H_{24}$	204	6.45	91	MS	[a]
8	1438	1492	aromadendrene	$C_{15}H_{24}$	204	6.29	161	MS	[a]
9	1377	1494	isoledene	$C_{15}H_{24}$	204	1.01	105	MS	[b]
10	1418	1495	(-)-β-caryophyllene	$C_{15}H_{24}$	204	0.88	69	MS	[a]
11	1511	1498	germacren-D-4-ol	$C_{15}H_{26}O$	222	3.52	161	I_R, MS	[c]
12	1499	1501	α-muurolene	$C_{15}H_{24}$	204	0.48	105	I_R, MS	[a]
13	1524	1509	δ-cadinene	$C_{15}H_{24}$	204	11.26	161	I_R, MS	[a]
14	1532	1512	cadin-1,4-diene	$C_{15}H_{24}$	204	0.80	119	I_R, MS	[a]
15	1542	1518	α-calacorene	$C_{15}H_{20}$	200	8.02	157	I_R, MS	[a]
16	1636	1523	δ-cadinol	$C_{15}H_{26}O$	222	3.71	161	MS	[d]
17	1531	1539	(-)-calamenene	$C_{15}H_{22}$	202	5.14	159	I_R, MS	[a]
18	1513	1548	(+)-cuparene	$C_{15}H_{22}$	202	8.99	132	I_R, MS	[b]

Mass spectra of unknown compound, m/z (int. %): 91(100), 105(98), 161(70), 133(70), 119(68), 79(68), 41(62), 67(52), 147(52), 55(40), 189(25). [a]Pino *et al.*, 2006; [b]Elías *et al.*, 1997; [c]Hamm *et al.*, 2004; [d]Hamm *et al.*, 2005.

Fragmentography and ten higher peaks intensity in MS analysis for each compound (Table 3) was applied and allowed to identify eighteen isomeric compounds. However, a detailed comparison between compounds [**4**] and [**5**] leads to propose that while its I_R has a little difference they present spectroscopic data that justify its structural diversity despite having the same molecular ion at *m/e* = 204 uma (Figure 3).

Analysis by HRCG-MS/EI of the EO led to identification of monoterpenes such as α-thujene, α and β-pinene, β-phellandrene, a monoterpenol known as β-linalool, some esters as bornil acetate, *trans* -pinecarvyl acetate and some sesquiterpenols among which are α-cadinol, α-bourbonenol and caryophyllene oxide; nevertheless, despite the great variety of compounds, EO is constituted by hydrocarbons (56.5%) and alcohols (24.7%) mainly (Table 4).

Table 4
Constituents of *C. polycarpus* leave essential oil by hydrodistillation

R_T	I_R theor.	I_R exp.	Compound name	MW	Area%	Base Peak	Id. criteria	I_R theor. Ref.
3.67	930	902	α-thujene	136	1.59	93	I_R, MS	[a]
3.81	940	948	α-pinene	136	15.9	93	I_R, MS	[a]
4.09	953	943	camphene	136	0.27	93	I_R, MS	[a]
4.56	1053	964	β-phellandrene	136	0.88	93	MS	[b]
4.64	980	943	β-pinene	136	10.75	93	I_R, MS	[a]
4.89	994	958	β-myrcene	136	0.49	41	I_R, MS	[c]
5.51	1017	998	α-terpinene	136	0.35	121	I_R, MS	[a]
5.71	1010	1042	m-cymene	134	2.82	119	I_R, MS	[d]
5.80	1020	1018	δ-limonene	136	0.78	68	I_R, MS	[b]
5.87	1039	1059	eucalyptol	154	1.53	43	I_R, MS	[b]

6.57	1062	998	γ-terpinene	136	5.01	93	MS	a
6.83	1189	1158	*cis*-α-terpineol	154	0.73	43	I_R, MS	e
7.73	1097	1081	β-linalool	154	3.20	71	I_R, MS	e
8.37	1141	1109	*trans*-2-menthenol	154	0.21	43	I_R, MS	f
8.89	1139	1131	L-*trans*-pinocarveol	152	0.76	92	I_R, MS	a
9.03	1132	1121	camphor	152	0.40	95	I_R, MS	b
9.07	1131	1136	*cis*-verbenol	152	0.66	94	I_R, MS	b
9.67	1228	1228	nerol	154	0.30	69	I_R, MS	a
10.05	1162	1137	terpinen-4-ol	154	0.72	71	MS	d
10.49	1172	1143	α-terpineol	154	0.70	59	MS	d
10.91	1265	1380	4-(1,2-dimethyl-cyclopent-2-enyl)-butan-2-one	170	0.64	95	MS	g
11.13	1227	1131	*trans*-3(10)caren-2-ol	152	0.56	109	MS	h
11.82	1234	1231	*o*-methylthymol	164	0.30	149	I_R, MS	a
11.96	1001	948	2-carene	136	1.13	93	I_R, MS	i
13.43	1275	1277	bornyl acetate	196	1.50	95	I_R, MS	c
14.11	---	1060	unknown 1	140	0.32	55	---	---
15.44	1086	1333	*p*-mentha-2 4(8)-diene	196	0.30	43	MS	j
15.95	---	1140	unknown 2	204	0.26	105	---	---
16.24	1377	1221	α-copaene	204	3.21	105	MS	e
16.52	1388	1344	β-bourbonene	204	0.47	81	I_R, MS	c, e
16.69	1351	1339	β-cubebene	204	0.33	161	I_R, MS	j
17.58	1418	1494	β-caryophyllene	204	6.44	93	MS	a, e, i
18.63	---	1579	unknown 3	204	0.75	93	---	---
18.84	1460	1386	L-alloaromadendrene	204	0.52	105	MS	a
19.34	1480	1515	germacrene D	204	2.64	161	I_R, MS	a
19.92	1511	1660	germacren-D-4-ol	222	1.32	161	MS	c
20.74	1524	1469	δ-cadinene	204	0.96	161	MS	a, c
21.32	1542	1547	α-calacorene	200	0.49	157	I_R, MS	a
21.93	1466	1410	dehydroaromadendrene	220	0.94	119	MS	k
22.18	1654	1580	α-cadinol	222	3.38	43	MS	a, e
22.36	1576	1569	sphathulenol	220	1.06	43	I_R, MS	a
22.47	1583	1507	caryophyllene oxide	220	2.90	43	MS	h
23.16	1604	1543	cedrenol	222	2.26	95	I_R, MS	l
23.48	1524	1537	L-calamenene	202	0.56	159	I_R, MS	c
23.71	No report	1387	α-vetivone	218	0.79	105	MS	---
24.31	---	1580	unknown 4	222	0.61	161	---	---
24.93	No report	1547	α-calacorenol	216	0.75	157	MS	---
25.21	No report	1646	ledene oxide (II)	220	3.57	43	MS	---
25.43	---	---	unknown 5	220	0.30	91	---	---
26.02	No report	1685	cycloisolongifol-8-ol	220	7.83	159	MS	---

Main constituents of Essential Oil	%
Total	95.1
Hydrocarbons	56.5
Alcohols	24.7
Esters	1.5
Ketones	1.8
Ethers	8.0
Unknown	2.5

[a]Pino *et al*., 2006; [b]Högnadóttir & Rouseff, 2003; [c]Hamm *et al*., 2004; [d]Cavalli *et al*., 2003; [e]Núñez-Arevalo *et al*., 2010; [f]Vardar-Ünlu *et al*., 2003; [g]Shivashankar *et al*., 2012; [h]Campeol *et al*., 2001; [i]Karioti *et al*., 2003; [j]Téllez *et al*., 2001; [k]Elías *et al*., 1997; [l]Choi, 2005.

The essential oil senescent leaves of *C. polycarpus* obtained by hydro distillation afforded 1.5% yield, and regarding to CO_2 supercritical extraction (SFE) was 0.1% with similar chemical composition but lower amount; its comparative analysis shows that HD is more efficient and cheap; the EO was analyzed by HRCG – MS ESI and compared in its constituents with C-2 non-polar fraction obtained by chromatographic methods (Blanco & Caicedo, 2010).

Besides, Tables 3 and 4 show that β-caryophyllene is the most abundant compound in both extracts as well as in other EO *Croton* species grown in Colombia such as *C. trinitatis* (Jaramillo-Colorado *et al*., 2016), *C. malambo* (Jaramillo *et al*., 2010), *C. bogotanus* (Núñez-Arévalo *et al*., 2010) has been analyzed. Several EO reports from other countries such as *C. flavens* (Sylvestre *et al.*, 2006), *C. urucurana* (Simionatto *et al.*, 2007), *C. cajucara* (Lopes *et al.*, 2000), *C. micradenus* and *C. myricaefolius* (Pino *et al*., 2005; Pino *et al.*, 2006) are reported in Table 5, which shows some common components besides to β-caryophyllene and its oxide or α and β pinene, more abudant compounds in these species.

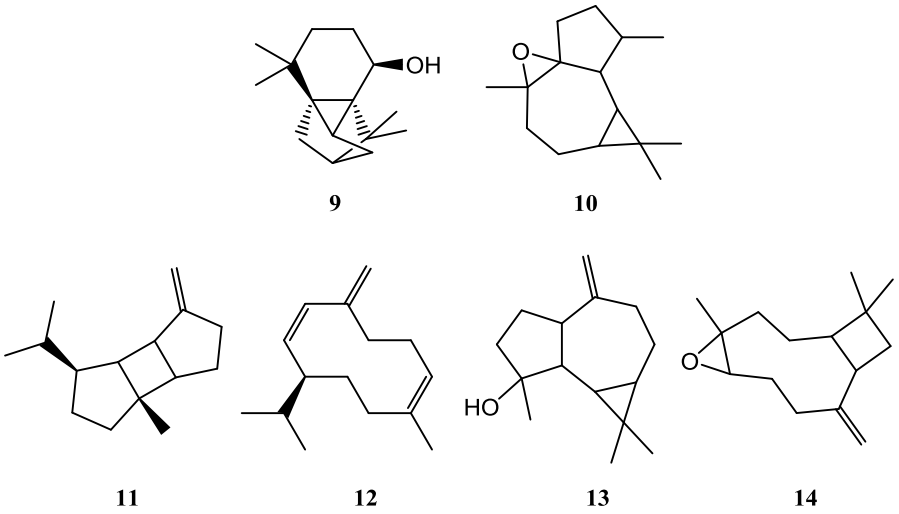
HRGC-MS analysis of EO shows that fresh leaves chemical composition of *C. polycarpus* are rich in monoterpenes like α-pinene (15.9%) and β-pinene (10.7%), sesquiterpenes such as β-caryophyllene (6.4%) and germacrene D (2.6%), sesquiterpenols as α-cadinol (3.4%), cedrenol (2.3%), cycloisolongifol-8-ol [**9**] (7.9%) and oxides such as caryophyllene oxide and ledene oxide II [**10**], with 2.9% and 3.6% respectively. Other alcohols are the 24.7% of EO. In Table 5, α- and β-pinene were the most abundant monoterpenes, which are common in most of analyzed species; however, their values are larger in *C. polycarpus* than in other species. Among most prevalent sesquiterpenes of the *Croton* EO are: α-copaene [**1**], β-bourbonene [**11**], β-caryophyllene [**2**], germacrene D [**12**], δ-cadinene [**4**] sesquiterpenols such as sphathulenol [**13**] and caryophyllene oxide [**14**] (Figure 4).

Table 5
Main constituents of essential oils from *Croton* species in comparison with *C. polycarpus.*

Compound name	Cp	Ct	Cm	Cb	Cf	Cu	Cc	Cmc	Cmy
α-thujene	1.59				0.27	0.1	<0.1		
α-pinene	15.9	0.4	0.4	3.2			0.6	5.3	3.9
camphene	0.27			<0.1			0.1	2.0	2.6
β-pinene	10.75	0.2	1.0	0.3			0.1	1.1	2.0
β-myrcene	0.49			0.6		0.2	<0.1	2.1	0.2
α-terpinene	0.35	0.4				0.1		<0.1	<0.1
δ-limonene	0.78	0.3		55.2		0.2	1.1	4.5	
eucalyptol	1.53	2.4				1.8	1.1		4.3
γ-terpinene	5.01				0.54	0.3	<0.1		0.1
β-linalool	3.20		5.6	0.7		0.3	41.2	34.9	1.2
trans-2-menthenol	0.21		0.2			0.1			
camphor	0.40	0.2							
α-terpineol	0.70			0.1		1.2		1.2	3.8
bornyl acetate	1.50		0.5			5.9	<0.1	3.3	
α-copaene	3.21		1.4	0.2	0.50	0.1	0.5		0.2
β-bourbonene	0.47	0.2	0.4	0.2	0.46		2.0		
β-caryophyllene	6.44	15.3	2.5	1.3	4.95		6.9	2.4	0.8
L-alloaromadendrene	0.52		0.6		1.21		0.4		
germacrene D	2.64	0.7	1.6	1.1	2.45		4.0	0.1	
germacren-D-4-ol	1.32				3.97				
δ-cadinene	0.96		0.6	0.3	2.31	0.8		0.2	0.2
α-calacorene	0.49					0.1		0.2	
dehydroaromadendrene	0.94				1.59				

α-cadinol	3.38	1.1		0.4	3.97			0.1	
sphathulenol	1.06	1.9	0.1			0.3	2.0		
caryophyllene oxide	2.90	2.5	0.6	0.2	3.55	0.4	1.8	2.8	8.8

Cp: *C. polycarpus*; **Ct**: *C. trinitatis*; **Cm**: *C. malambo*; **Cb**: *C. bogotanus*; **Cf**: *C. flavens*; **Cu**: *C. urucurana*; **Cc**: *C. cajucara*; **Cmc**: *C. micradenus*; **Cmy**: *C. myricaefolius*.



9 **10** **11** **12** **13** **14**

Figure 4
Main compounds in analyzed *Croton* species

From polar fraction of *C. polycarpus* leaves three flavonols derivatives were isolated, known as quercetin and their glycosides quercitrin (quercetin-3-O-α-L-rhamnoside) and rutin (quercetin-3-rutinoside). Quercetin and rutin flavonoids present an EC_{50} of 47 μg/mL and 64 μg/mL respectively, against *C. quinquefasciatus* mosquitoes larvae, having a promissory larvicidal activity; nevertheless, the quercitrin doesn't give notable bioactivity in this specific LAB, although its antioxidant activity is widely known (Nuñez-Arevalo, 2005). Moreover, these compounds have showed the highest antioxidant activity, (Zhang *et al*., 2014) and are useful ingredients in nutraceutics and this preliminary study of *C. polycarpus* is a contribution to the knowledge of our native species. (Sintayehu *et al*., 2012; Bose *et al*., 2013).

CONCLUSIONS

Herein, are reported preliminary results about the presence of a group of sesquiterpenoids and a flavonol and their glycosides characterized by HRGC-MS and NMR from the outer bark and leaves of *C. polycarpus* tree. For compounds derived of decaline nucleus whose structures depend on different substituent groups or double bond positions, like in δ-cadinene and cadina-1,4-diene, could be seen the functional diversity and stereochemical arrangements joint with the efficiency of the selected chromatographic systems to separate isomers and compounds with similar structures, that have potential as pedagogical strategy and other applications in formative programs. This work is part of a research project that studies the chemical composition and biological activity of native plants and other fractions are under current investigation.

Quercetin: MeOHd_4. ^{1}H-NMR (δ in ppm, *J* in Hz) 12.76 (1H, s, C5-OH), 6.24 (1H, d, *J*=2.0, C6-H), 6.46 (1H, d, *J*=2.0, C8-H), 7.51 (1H, d, *J*=2.1, C2'-H), 6.95 (1H, d, *J*=8.3, C5'-H), 7.39 (1H, dd *J*= 8.3, 2.0, C6'-H).

Quercitrin: $Me_2CO d_6$. ^{1}H-NMR (δ in ppm, *J* in Hz) 12.64 (1H, s, C5-OH), 6.26 (1H, d, *J*=2.0, C6-H), 6.47 (1H, d, *J*=2.0, C8-H), 7.51 (1H, d, *J*=2.3, C2'-H), 6.95 (1H, d, *J*=8.2, C5'-H), 7.37 (1H, dd, - *J*=8.2, 2.3, C6'-H), 5.50 (1H, s broad, C1''-H), 4.23 (1H, d,

J=1.6, C2''-H), 3.75 (1H, dd, *J*=9.2,3.2, C3''-H), 3.35 (1H, t, *J*=9.2, C4''-H), 3.42 (1H, dd, *J*=9.4, C5''-H), 0.91 (3H, d, *J*=6.4, C6''-CH_3); ^{13}C -RMN δ in ppm) 158.9 (C-2), 136.8 (C-3), 180.3 (C-4), 165.9 (C-5), 95.5 (C-6), 166.0 (C-7), 100.5 (C-8), 159.3 (C-9), 106.7 (C-10), 123.5 (C-1'), 117.0 (C-2'), 146.8 (C-3'), 150.0 (C-4'), 117.1 (C-5'), 123.8 (C-6'), 103.7 (C-1''), 73.0 (C-2''), 72.4 (C-3''), 72.3 (C-4''), 70.8 (C-5''), 18.8 (C-6'').

Rutin: MeOHd_4. ^{1}H-NMR (δ in ppm, *J* in Hz) 12.7 (1H, d, *J*=2.33, C5-H), 6.27 (1H, d, *J*=2.0, C6-H), 6.49 (1H, d, *J*=2.0, C8-H), 7.55 (1H, d, *J*=2.0, C2'-H), 7.00 (1H, d, *J*=8.0, C5'-H), 7.46 (1H, dd, *J*=8.0,2.0, C6'-H), 5.21 (1H, d, *J*=8.2, C1''-H), 4.91 (1H, d, *J*=2.0, C1'''-H), 1.25 (1H, d, *J*=6.0, C6'''-CH_3).

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