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Volatile Secondary Metabolites in Cascarillo (*Ocotea caparrapi* (Sandino-Groot ex Nates) Dugand - Lauraceae)

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Abstract: In this work, the chemical analysis of the volatile fractions obtained by simultaneous distillation-extraction of aerial parts of a species of *Ocotea* aff. *O. caparrapi* known as “Cascarillo” was carried out by GC-MS. The main components identified were: α -pinene (23.9 %) and β -pinene (14.7 %), in the resin; α -terpineol (26.6 %) and methyleugenol (14.7 %), in the leaves; elemicin (31.3 %) and methyleugenol (31.1 %), in the branch bark; myristicin (35.4-46.1 %), terpinen-4-ol (14.9-26.3 %), and α -terpineol (17.5-21.3 %), in stem bark. A high content of monoterpene hydrocarbons (82.6 % and 51.8 %) were found in the resin and leaves; and propenylbenzenes (66.5 %), in the branch bark. Oxygenated monoterpenes (44.5-57.3 %) and propenylbenzenes (52.3-38.7 %) constituted the stem bark. The identification of methyleugenol, elemicin and myristicin, in the volatile fractions, allowed us to establish a relationship between the *Ocotea* sp. of our study with *O. caparrapi* and *O. cymbarum*.

Key words: *Ocotea*; Cascarillo; volatile fractions; propenylbenzenes; SDE-GC-MS.

Introduction

The Lauraceae family has *ca.* 55 genera and 3000 species, and is constituted by trees and shrubs, which grow in wet and dry tropical forests (0 m to 4000 m). It is widely distributed in America, Asia and Australia, and with a little representation in Africa ¹⁻³. This family is important from the economic point of view by its woods, resins, essential oils (Camphor - *Cinnamomum camphora*), spices (cinnamon - *Cinnamomum zeylanicum*; laurel - *Laurus nobilis*), and, fruits (avocado - *Persea americana*) ¹.

Ocotea is the most diverse and abundant genus of the family, with about 350 species mainly distributed in the neotropics, from Mexico to Argentina, and also is found in Africa (Madagascar

and Canary Islands) ²⁻⁵. This genus includes timbers, which for its fragrant features are a great source of essential oils (EO) constituted by terpenes, phenylpropanoids (*e.g.*, safrole, asaricin, myristicin, phenols, aldehydes) or related compounds (*e.g.*, caparratriene, caparrapi oxide) ^{4, 6-8}. Additionally, compounds belonging to classes: flavonoids, lignanes, neolignanes, and alkaloids (benzylisoquinolinics, aporfines) are found in the *Ocotea* spp. ^{3, 9-12}. However, it has been carried to endangered conditions due to the deforestation and unsustainable exploitation ^{4, 5, 13}.

Some *Ocotea* species (*O. quixos*, *O. puchury-major*, *O. longifolia*, *O. bullata*, *O. opifera*, *O. paulii*) have pharmacological or therapeutic properties as anti-rheumatic, analgesic, anesthetic,

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antidiarrheal, anti-inflammatory, antithrombotic, antiplatelet, antioxidant, antimicrobial, anti-abscesses, gastroenteric, purgative, tonic, headache, nervous disorders, sedative and appetizer. Other species, *O. pretiosa*, *O. sassafras*, *O. caudata*, *O. odorifera*, *O. caparrapi* and *O. cymbarum*, have a recognized position in the market, specifically in perfumery. And, the others, *O. bofo*, *O. austinii* and *O. corymbosa*, have been used as flavoring, furniture and construction^{4, 5, 7, 9-19}.

In this work, we carried out the chemical analysis of an *Ocotea aff. O. caparrapi*, called "Cascarillo". Gas Chromatography coupled to Mass Spectrometry (GC-MS) was used for the analysis the volatile fractions of this species. Simultaneous Distillation-Extraction technique (SDE) was used for obtaining volatile fractions of different parts of the plant material (resin, leaves, and branch and stem barks). The importance of this work lies in the commercial potential of this species for the production of essential oils, resins and wood; in addition, because for this endangered species there are few documented studies for Colombia^{6, 20-25}.

Methods

Reagent

The solvents employed were dichloromethane (ACS grade, Riedel-de Haën) and distilled water. Anhydrous sodium sulfate (Analytical grade, Biopack) was used as a dehydrating agent.

Plant material

"Cascarillo" (*Ocotea aff. O. caparrapi*, N° 0.38 of *Orinoquía's Science Research Group*) was collected in "El Porvenir" village, municipality of Toledo, Departamento de Norte de Santander (Colombia). Between 100 g and 1000 g of each part of the plant material was collected. The resin was obtained by drilling the stem of the tree to the marrow. The preliminary taxonomic identification of botanical sample was performed by Gerardo Aymard Antonio Corredor (UNELLEZ - Guanare - Venezuela), expert in Venezuelan forest. The plant collection was made under Resolution No. 739 of July 8, 2014, conferred by the Agencia Nacional de Licencias Ambientales (ANLA).

Simultaneous distillation - extraction solvent (SDE)

The volatile fractions of the different parts of fresh plant were isolated using a Likens & Nickerson microscale apparatus, modified by Godefroot *et al.*²⁶, for high density solvents. 10 g of vegetal material were used, the extraction solvent was dichloromethane (2 mL), and the process was carried out during 2 h. The extracts were dehydrated with anhydrous sodium sulfate. For the case of resin, 50 µL of pure resin were dissolved in 500 µL of acetone. Finally, 1 mL of the extracts was analyzed by GC-MS.

Gas chromatography-Mass spectrometry (GC-MS)

The separation and analysis of the components present in the SDE extracts were carried out in a Trace 1310 gas chromatograph coupled to a mass selective detector ISQ (Thermo Fisher Scientific, Inc.), with split/splitless inlet (ratio split 20:1), automatic injection system (AI/AS 1310 Thermo Scientific). Rxi®-1ms column (30 m x 0.25 mm I.D. x 0.5 µm d_p), with stationary phase of 100 % dimethyl-polysiloxane, was used for the separation. Helium (99.999 %) was the carrier gas (constant flow, 1.0 mL/min). Oven temperature programming was from 50°C (5 min) to 150°C (10 min) at a rate of 2°C/min, a second rate of 3°C/min to 250°C (5 min). Ion source and transfer line temperatures were maintained at 230°C and 250°C, respectively. Mass spectra were obtained by electron impact (EI, 70 eV) using quadrupole mass analyzer. Total ion currents (TIC) were acquired by full scan mode, with a mass range of m/z 40-350. Chromatographic and spectroscopic data were processed using by Thermo Xcalibur™ (Version 2.2 SP1.48, Thermo Fisher Scientific, Inc.) and AMDIS (Automated Mass Spectral Deconvolution and Identification System, 13 May 2011, Build 130.53, Version 2.70) softwares.

Linear temperature-programmed retention indices were calculated from the data of a homologous series of saturated aliphatic hydrocarbons (C_7 - C_{35}) and analyzed under the same conditions that the extracts. The secondary metabolites were identified by comparing their mass spectra with those of the databases (NIST11, NIST Retention

Index and Wiley9), and with linear retention indices reported in the existing literature ²⁷⁻²⁹.

Results and discussion

Table 1 contains the identified compounds from the volatile fractions (VF) isolated of the resin, leaves, and branch and stem barks of *Ocotea aff. O. caparrapi*, along with their relative amounts and retention indices (calculated and literature). Figure 1 shows the total ion currents (TIC) obtained by GC-MS of each VF analyzed.

According to the Table, α -pinene (23.9 %), β -pinene (14.7 %), p-cymene (7.8 %), and α -terpineol (7.3 %) were the main constituents of the resin sample; linalool (8.6 %), terpinen-4-ol (8.7 %), α -terpineol (26.6 %), methyleugenol (14.7 %), and bicyclogermacrene (6.7 %), were identified in the leaves sample; also, in the branch bark sample were found α -terpineol (12.6 %), methyleugenol (31.1 %), elemicin (31.3 %) and myristicin (2.9 %), as the most abundant compounds; on the other hands, the stem barks of the

Table 1. Chemical composition by GC-MS, for resin and VF of leaves, and branch and stem barks of *Ocotea aff. O. caparrapi*

No.	Compound	Relative amount, %					I_R	
		Resin	Leaves	Branch bark	Stem bark ¹	Stem bark ²	Calculated	Literature
1	α -Pinene	23.9	-	-	-	-	936	930
2	α -Fenchene	0.4	-	-	-	-	942	940
3	Camphene	3.5	-	-	-	-	943	943
4	β -Citronellene	0.4	-	-	-	-	944	944
5	Sabinene	0.2	-	-	-	-	965	964
6	β -Pinene	14.7	-	-	-	-	969	970
7	Myrcene	1.1	-	-	-	-	983	981
8	p-Menth-2-ene	0.7	-	-	-	-	990	982
9	p-Cymene	7.8	-	-	tr	-	1008	1011
10	1,8-Cineole	3.9	0.1	-	0.2	0.2	1016	1020
11	Limonene	4.7	-	-	-	-	1018	1020
12	γ -Terpinene	0.9	-	-	-	-	1046	1047
13	<i>cis</i> -Sabinene hydrate	-	0.6	-	-	-	1048	1041
14	Fenchone	tr	-	-	0.5	0.9	1062	1066
15	Terpinolene	0.7	-	-	-	-	1075	1078
16	<i>trans</i> -Sabinene hydrate	-	1.2	-	-	-	1077	1051
17	Linalool	0.2	8.6	4.6	0.2	-	1082	1082
18	Fenchol	2.3	-	-	0.1	0.2	1092	1100
19	α -Campholenal	0.2	-	-	-	-	1098	1102
20	<i>cis</i> p-Menth-2-en-1-ol	tr	0.4	-	-	-	1101	1106
21	Camphor	0.7	-	-	2.4	3.2	1112	1115
22	<i>trans</i> -Pinocarveol	0.9	-	-	-	-	1115	1117
23	<i>cis</i> -Verbenol	0.2	-	-	-	-	1118	1127
24	<i>trans</i> -Verbenol	2.0	-	-	-	-	1122	1122
25	<i>trans</i> p-Menth-2-en-1-ol	-	0.3	-	-	-	1128	1123
26	Camphene hydrate	-	-	-	0.2	0.1	1124	1128
27	2,2,4-Trimethyl-3-cyclopentene-1-ethanol	-	-	-	0.2	0.3	1140	NR
28	δ -Terpineol	-	3.8	0.6	-	-	1140	1142

table 1. (continued).

No.	Compound	Relative amount, %					I _R	
		Resin	Leaves	Branch bark	Stem bark ¹	Stem bark ²	Calculated	Literature
29	Pinocamphone	-	-	-	0.2	-	1141	1140
30	Borneol	2.5	-	0.2	0.2	0.3	1142	1148
31	Unknown	-	-	-	0.4	0.1	1144	-
32	Terpinen-4-ol	1.3	8.7	3.2	14.9	26.3	1158	1160
33	p-Cymen-8-ol	1.3	-	-	-	-	1158	1152
34	Myrtenal	0.2	-	-	-	-	1161	1163
35	α-Terpineol	7.3	26.6	12.6	17.5	21.3	1169	1172
36	Myrtenol	1.1	-	-	-	-	1172	1174
37	Verbenone	-	-	-	3.5	1.8	1173	1191
38	cis-Piperitol	-	0.2	-	-	-	1174	1179
39	Borneol formate	-	-	-	0.9	0.7	1180	1208
40	trans-Piperitol	-	0.3	-	-	-	1184	1189
41	trans-Carveol	0.3	-	-	-	-	1191	1195
42	2-Hydroxy-1,8-cineole	-	-	-	0.7	0.3	1192	1212
43	Fenchol acetate	0.2	-	-	-	-	1201	1207
44	Isoborneol formate	0.2	-	-	-	-	1203	1222
45	Nerol	-	0.5	0.2	-	-	1206	1209
46	2,5-Bornanedione	-	-	-	0.4	-	1207	1264**
47	cis-Myrtenol	-	-	-	0.3	tr	1214	1254*
48	Piperitone	-	-	-	1.0	1.0	1218	1228
49	trans-Myrtenol	-	-	-	0.5	-	1233	1254
50	Safrole	-	-	-	0.3	-	1256	1261
51	p-Cymen-7-ol	-	-	-	0.3	-	1258	1266
52	Borneol acetate	1.5	0.2	-	-	-	1264	1273
53	Unknown	0.8	-	-	-	-	1271	-
54	Unknown	0.5	-	-	-	-	1274	-
55	Unknown	0.2	-	-	-	-	1277	-
56	Terpinen-4-ol acetate	1.1	-	-	-	-	1279	1281
57	Unknown	0.6	-	-	-	-	1286	-
58	Unknown	0.2	-	-	-	-	1291	-
59	α-Terpineol acetate	4.3	0.9	-	0.2	0.7	1328	1322
60	Carvyl acetate derivative	1.0	-	-	-	-	1337	-
61	Nerol acetate	-	0.2	-	-	-	1341	1342
62	Methyleugenol	-	14.7	31.1	0.6	0.2	1366	1378
63	β-Bourbonene	-	0.2	-	-	-	1374	1386
64	β-Elemene	-	0.3	-	-	-	1380	1387
65	β-Caryophyllene	-	4.4	0.4	-	-	1405	1420
66	Nerylacetone	-	-	-	0.2	0.3	1424	1427
67	Unknown(178 molecular ion)	-	-	-	0.4	0.2	1431	-
68	α-Humulene	-	0.5	-	-	-	1438	1454
69	4,5-di-epi-Aristolochene	-	0.3	-	-	-	1454	1467

table 1. (continued).

No.	Compound	Relative amount, %					I _R Calculated	I _R Literature
		Resin	Leaves	Branch bark	Stem bark ¹	Stem bark ²		
70	Germacrene D	-	2.4	0.5	-	-	1464	1480
71	4-epi-Cubebol	-	0.3	-	-	-	1476	1489
72	Bicyclogermacrene	-	6.7	-	-	-	1479	1482
73	Myristicin	0.8	-	2.9	46.1	35.4	1485	1482
74	γ-Amorphene	-	0.5	0.2	-	-	1495	NR
75	δ-Cadinene	-	0.3	0.5	-	-	1504	1514
76	Elemicin	-	5.2	31.3	5.6	3.1	1512	1518
77	<i>cis</i> -3-Hexenol benzoate	-	0.3	-	-	-	1532	1540
78	<i>trans</i> -Nerolidol	5.3	-	-	1.7	3.5	1538	1548
79	Spathulenol	-	5.7	0.2	-	-	1545	1564
80	Unknown	-	-	0.2	-	-	1547	-
81	Caryophyllene oxide	-	2.4	1.8	0.2	-	1550	1575
82	Methoxyeugenol	-	-	1.2	-	-	1551	1560
83	Globulol	-	0.2	-	-	-	1554	1570
84	Unknown	-	0.3	-	-	-	-	-
85	Viridiflorol	-	0.2	1.1	-	-	1562	1592
86	Guaiol	-	-	0.8	-	-	1569	1582
87	Humulene epoxide II	-	0.5	0.6	-	-	1574	1596
88	1-epi-Cubenol	-	-	0.4	0.1	-	1600	NR
89	allo-Aromadendrene epoxide	-	1.3	-	-	-	1606	NR
90	τ-Cadinol	-	-	0.7	-	-	1610	1628
91	τ-MuuroloI	-	0.3	0.7	-	-	1611	1627
92	δ-Cadinol	-	-	0.4	-	-	1614	1640
93	Unknown	-	0.4	0.3	-	-	1619	-
94	α-Cadinol	-	0.3	1.8	-	-	1622	1637
95	Unknown	-	-	0.3	-	-	1636	-
96	Shyobunol	-	-	1.3	-	-	1658	NR
97	Unknown	-	-	-	0.3	-	1781	-

Stem bark¹: alive threeStem bark²: death three (without branch)I_R: Calculated o experimental, and literature retention indices²⁷⁻²⁹

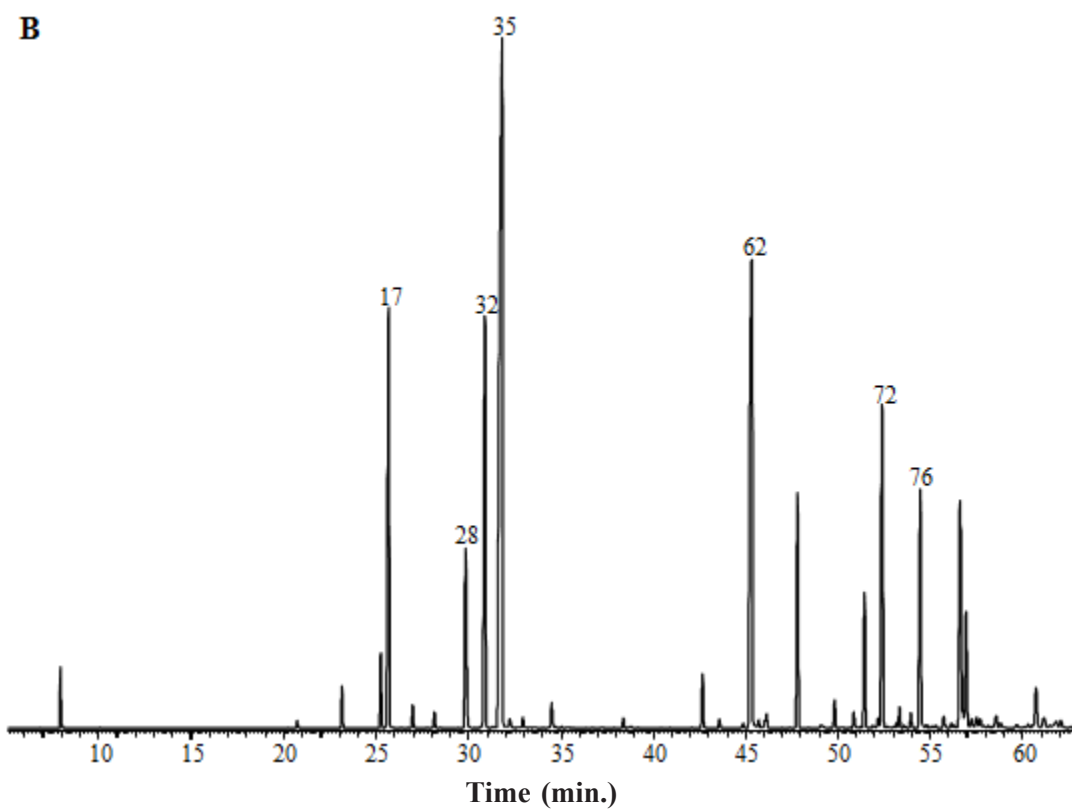
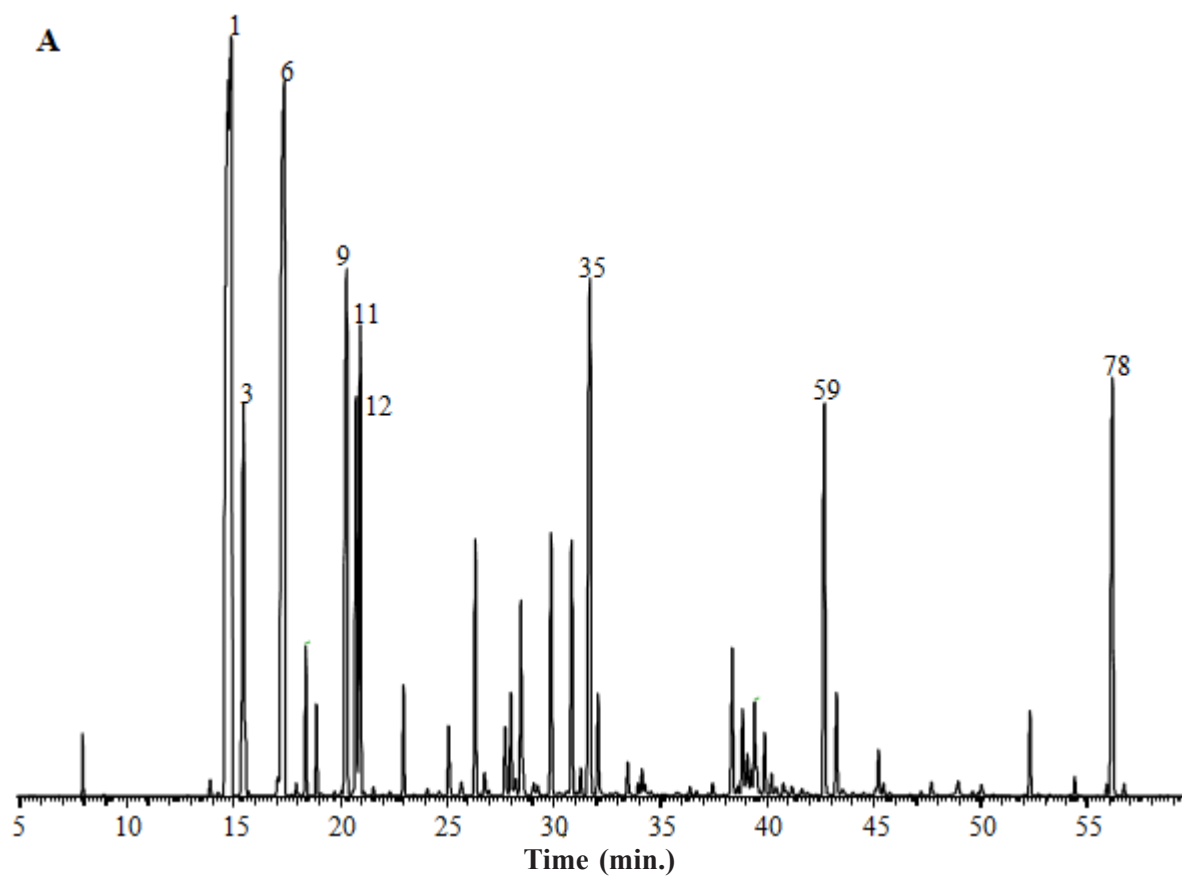
tr: Trace (<0.1 %)

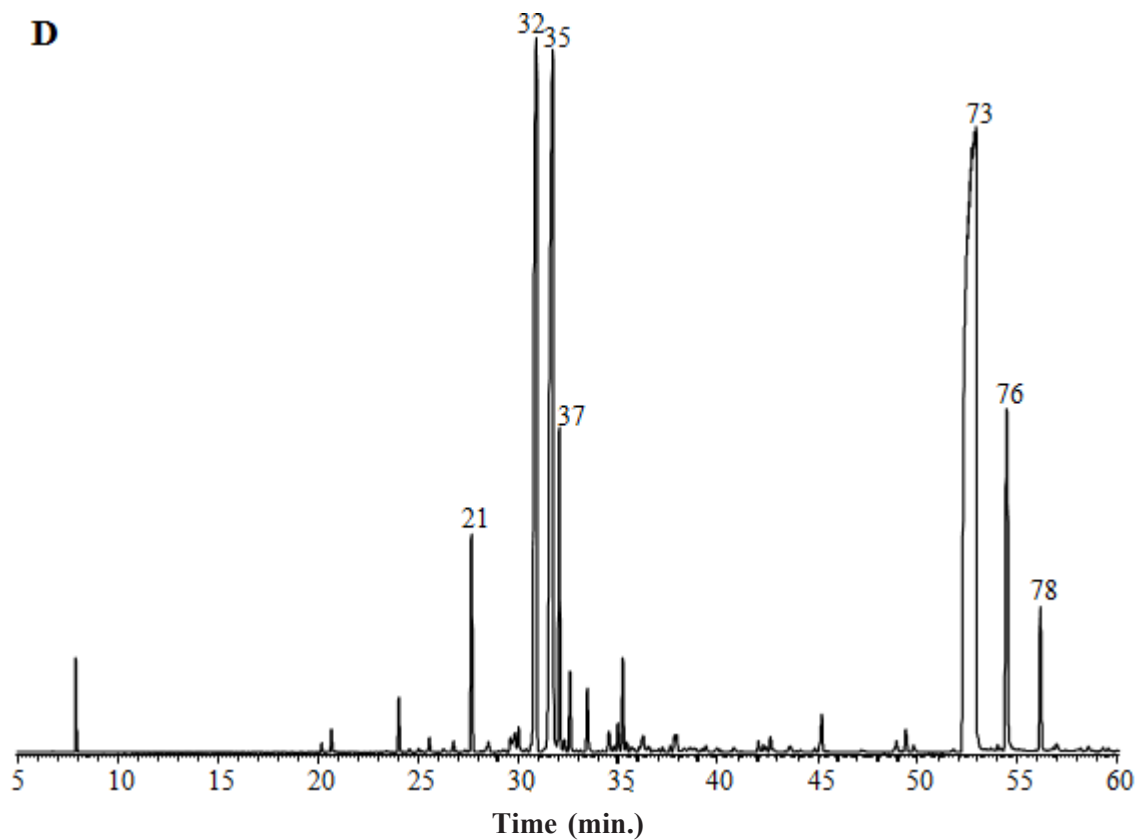
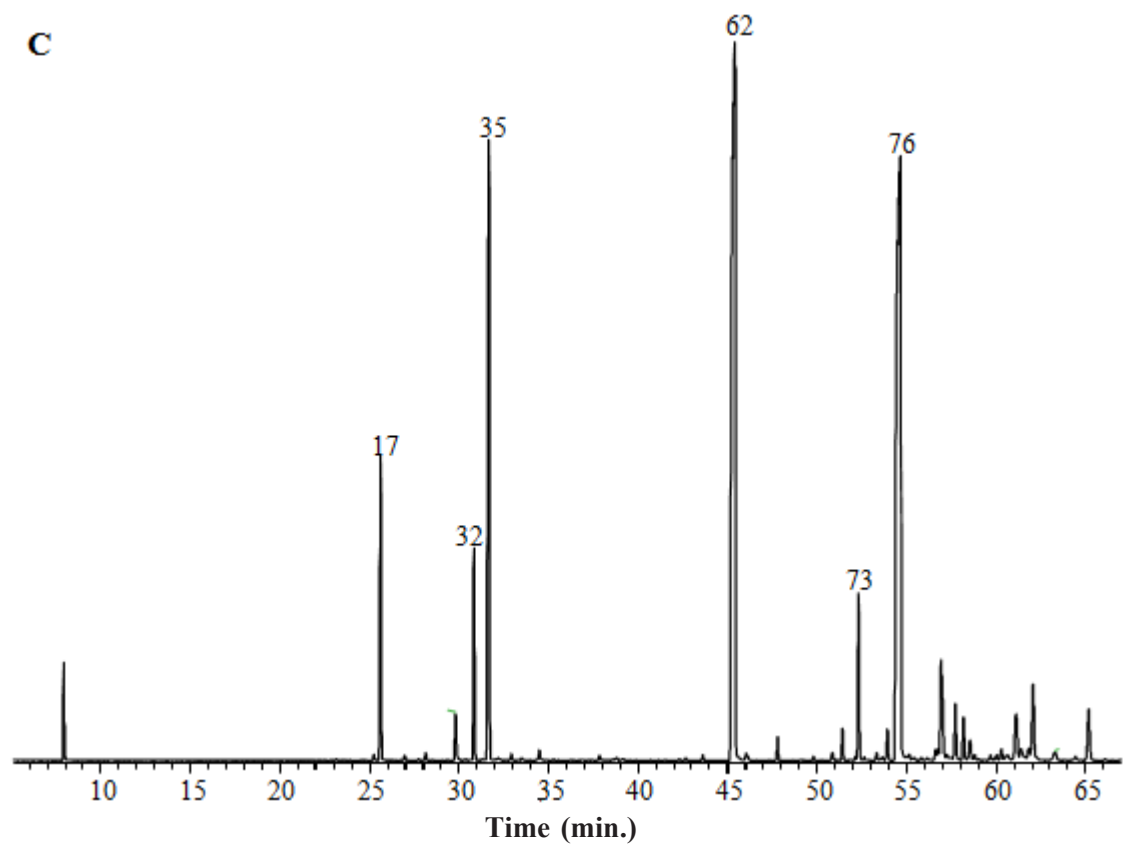
NR: not reported

alive and death trees were characterized respectively by terpinen-4-ol (14.9 % and 26.3 %), α-terpineol (17.5 % and 21.3 %), and myristicin (46.1 % and 35.4 %).

In accordance with Figure 2, the resin of *Ocotea aff. O. caparrapi* was represented by a high percentage of monoterpenes (hydrocarbons and

oxygenated, ~ 51.2 % and 31.4 %); sesquiterpenes and propenylbenzenes contributed with a low percentage (5.3 % and ~ 0.8 %). The leaves were characterized by oxygenated monoterpenes (~ 51.8 %), in conjunction with propenylbenzenes (19.8 %) and sesquiterpenes (hydrocarbons and oxygenated, ~ 15.5 % and 11.3 %). The branch





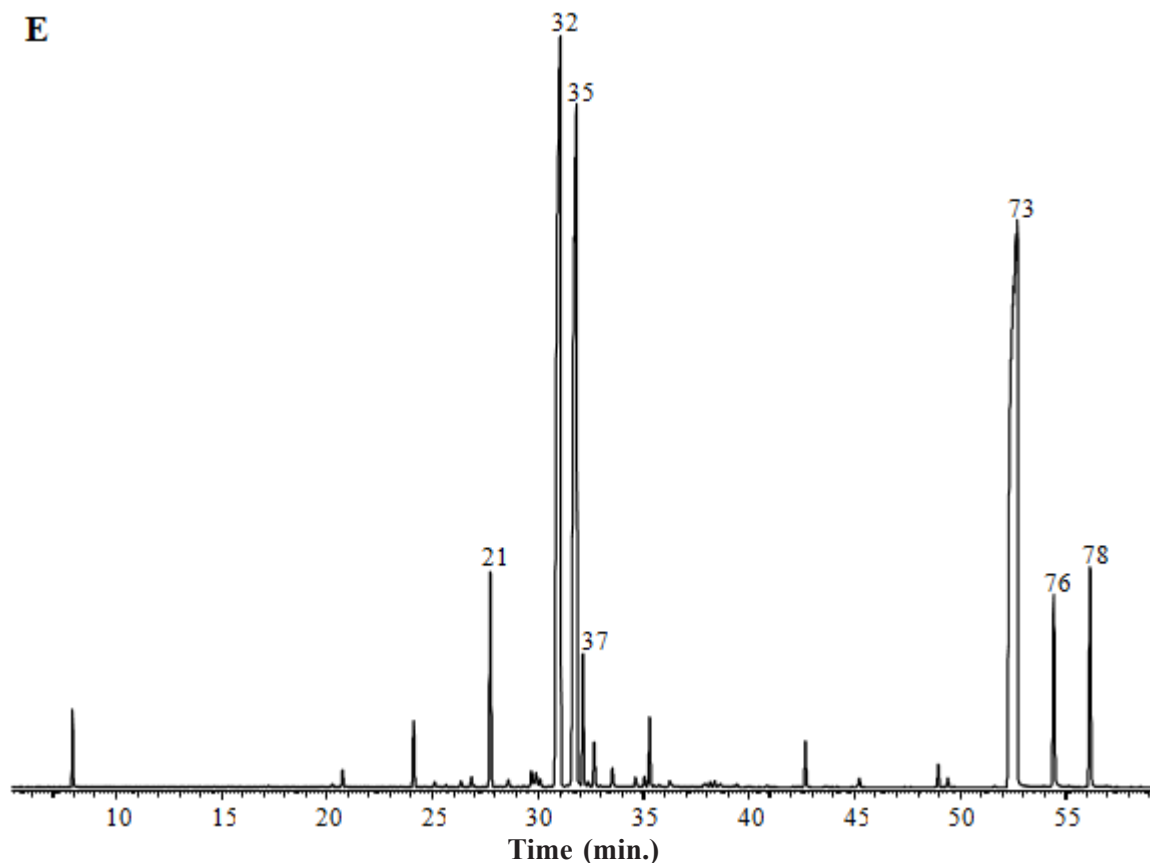


Figure 1. TIC obtained by GC-MS analysis, from *Ocotea aff. O. caparrapi*. A. Resin; and VF of B. Leaves; C. Branch bark; D. fresh stem bark, and E. death stem bark

bark was constituted by propenylbenzenes (66.5 %), oxygenated monoterpenes (~ 21.2 %), and sesquiterpenes (hydrocarbons and oxygenated, ~ 1.6 % and 9.8 %). Finally, the stem barks of alive and death trees were represented by a high percentage of oxygenated monoterpenes (~ 44.5 % and 57.3 %), and propenylbenzenes (methyleugenol - 0.6 % and 0.2 %, myristicin - 46.1 % and 35.4 %, elemicin - 5.6 % and 3.1 %).

The specific chemical composition of the volatile metabolites determined in the different parts of *Ocotea aff. O. caparrapi*, of interest, differed of the reports found about the others Colombian *Ocotea* spp. (*O. caparrapi*, *O. longifolia*, *O. macrophylla*), which were rich in nerolidol (92 %), caparrapi oxide (43 %), α -terpinolene (81 %), spathulenol (16 %), and γ -muurolene (15 %) ^{6,22-24}. However, the composition of the resin has some similarities with the reported by Delgado *et al.* ²⁵, from the oil of Cascarillo acquired in the market of the city of Arauca, (Colombia). α -Pinene (47.2

%), camphor (21,4 %), β -phellandrene (8.1 %), β -myrcene (7.2 %), and β -pinene (4.2 %) were the main components reported by Delgado *et al.* ²⁵, who indicated that the plant known as Cascarillo could be related to the species *O. barcellensis* (synonymy of *O. cymbarum*).

Myristicin (35.4-46.1 %), and methyleugenol (0.2-0.6 %), determined in the VF of stem bark from *Ocotea aff. O. caparrapi* were also detected in the EO from wood of *O. caparrapi* studied by O. González ³⁰. In addition, nerolidol, caparrapi oxide, and caparrapiol, have also been detected as biomarker compounds for this species ^{4, 6, 20, 31}.

A distinctive constituent of some *Ocotea* spp., *e.g.*, *O. puchury-major*, and *O. pretiosa* (*Sassafras albidum*), has been safrole, which is found in high percentage in these plants (above 30 %) ^{5, 19, 32-34}. However, in the species of interest (*Ocotea aff. O. caparrapi*), safrole was determined only at trace level in the volatile fraction of

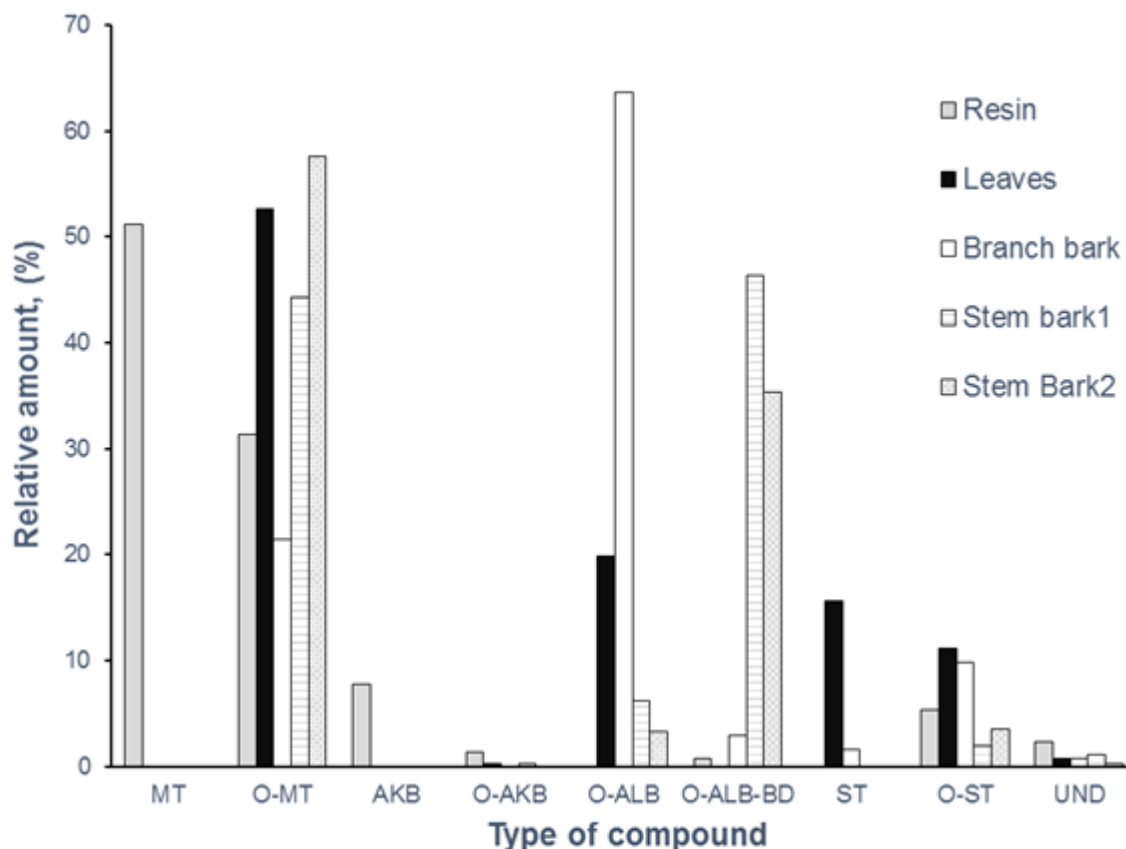


Figure 2. Types of compounds present in resin, and VF of leaves, and branch and stem barks of *Ocotea aff. O. caparrapi*.

Monoterpenes (MT), Oxygenated monoterpenes (O-MT), Alkyl benzenes (AKB), Oxygenated alkyl benzenes (O-AKB), Propenylbenzenes (Oxygenated allyl benzenes (O-ALB), Oxygenated allyl benzenes-benzodioxole (O-ALB-BD)), Sesquiterpenes (ST), Oxygenated sesquiterpenes (O-ST), not identified compound (UND), (Stem bark1: alive three; Stem bark2: death three (without branch)).

the stem bark (0.3 %).

Moreover, methyleugenol was the main component of leaves and branch bark for *Ocotea aff. O. caparrapi* (14.7 % y 31.1 %); nevertheless, the ethanol extract of wood from *O. cymbarum* contained some eugenol derivatives as dehydroeugenol, mono-*o*-methyldehydrodieugenol and dehydrodieugenol, which were the majority components^{35, 36}.

Chemical composition reported for *O. quixos* was different of *Ocotea* sp. under study; thus, the essential oil of the flower calices from *O. quixos* was constituted by *trans*-cinnamaldehyde (28 %), cinnamic acid methyl ester (22 %), β -caryophyllene (15 %), acetic acid cinnamyl ester (11 %), and sabinene (8 %). This species has been evaluated about the antiplatelet, anticoagulant, anti-inflammatory, antioxidant, antimicrobial and anti-

fungal properties^{7, 8, 13, 14, 37, 38}.

On the other hand, methyleugenol and elemicin, the main components detected for branch bark of *Ocotea aff. O. caparrapi* were also identified in other species such as *Laurus nobilis* (Lauraceae), *Asiasarum sieboldi* and *Asarum cordifolium* (Aristolochiaceae), *Artemisia dracunculul* (Asteraceae), *Pseuduvaria mulgraveana* (Annonaceae), *Croton malambo* (Euphorbiaceae) and *Ocimum campechianum* (Lamiaceae)³⁹⁻⁴⁴. Likewise, elemicin and myristicin were found in *Ferula heuffelii*, *Heracleum pastinacifolium*, and *Heracleum transcaucasicum* (Apiaceae), *Piper krukoffii* (Piperaceae), and *Aristolochia acutifolia* (Aristolochiaceae)⁴⁵⁻⁴⁹.

Finally, the pharmacological properties and uses of the most abundant compounds identified in the

volatile fractions from *Ocotea aff. O. caparrapi* have been previously determined and reported in the scientific literature. Thus, the monoterpenoids terpinen-4-ol and α -terpineol are mildly irritating and non-sensitising, diuretics, antibacterial, sedatives, antiseptics and antiallergics. These compounds are used as ingredients in the flavor, perfumery and food industries⁵⁰⁻⁵².

The 2-propenylbenzene derivatives methyleugenol and elemicin are “suspected” of genotoxicity and carcinogenicity. However, methyleugenol has antinociceptive, antiseptic and insect attractant properties. This compound has been used as ingredient in the flavor and perfumery industries. Meanwhile, elemicin has insecticide, antifeedant and hypotensive activities and is used as starting material for the synthesis of antibacterial drug (trimethoprim), and in topic analgesic formulations⁵³⁻⁶².

The last compound, myristicin, a safrole derivative with a methoxy group attached at carbon 4, has interesting properties such as insecticide, wound healing, anticancer and psychedelic activities, and hepatoprotective function; and this compound has application as food flavoring. Some synthetic analogs from myristicin resulted more potent than the natural product podophyllotoxin⁶²⁻⁶⁹.

Conclusions

In conclusion, this work is the first report on the chemical composition of the resin and the volatile fractions of aerial parts of “Cascarillo”, an *Ocotea* sp. from Toledo (Norte de Santander, Colombia).

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Furthermore, the high content of methyleugenol (31 %), elemicin (31 %) and myristicin (35 % - 46 %) determined in the branch/stem barks allowed us to estimate the potential of this species as a possible source of raw materials (compounds) for industries related (flavor, perfumery and food). Finally, the *Ocotea* sp. of our study would have unique features due to the presence and content of elemicin (without previous reports), which are attributable to the exceptional ecological conditions of the geographical area of collection.

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Abbreviations

Agencia Nacional de Licencias Ambientales (ANLA); Essential Oils (EO); Gas Chromatography coupled to Mass Spectrometry (GC-MS); Simultaneous Distillation-Extraction Technique (SDE); Total Ion Currents (TIC); Volatile Fractions (VF).

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