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Chemical composition and antimicrobial activity of the essential oil of the leaves of *Ocotea caudata* (Nees) Mez (Lauraceae) from Colombia

[Composición química y actividad antimicrobiana del aceite esencial de las hojas de *Ocotea caudata* (Nees) Mez (Lauraceae) de Colombia]

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Abstract: *Ocotea* is a genus that belongs to the Lauraceae family, which has about 56 species, distributed in Asia, Africa and mainly in America. The aim of this work was to identify the chemical composition of the essential oil from leaves of *Ocotea caudata* collected from Colombia. The chemical composition of the oil was determined by gas chromatography-mass spectrometry (GC-MS), being described for the first time. Thirty nine compounds (corresponding to 92.7% of the oil) were identified. The major constituents were germacrene D (55.8%), bicyclogermacrene (8.0%), β -caryophyllene (4.6%) and β -bourbonene (2.3%). Also the antibacterial activity of the oil was evaluated against two Gram (+) and two Gram (-) bacteria showing that the oil exhibited moderated activity against Gram (+) bacteria.

Keywords: Essential oils, Lauraceae, *Ocotea caudata*, GC-MS, germacrene D

Resumen: *Ocotea* es un género perteneciente a la familia Lauraceae, que contiene cerca de 56 especies, distribuidas en Asia, Africa y principalmente América. El objetivo de este trabajo fue identificar la composición química del aceite esencial de las hojas de *Ocotea caudata* colectadas en Colombia. La composición química del aceite fue determinada por cromatografía de gases-espectrometría de masas (GC-MS), siendo descrita por primera vez. Se identificaron treinta y nueve compuestos (correspondientes al 92.7% del aceite). Los componentes mayoritarios fueron germacreno D (55.8%), bicilogermacreno (8.0%), β -cariofileno (4.6%) y β -bourboneno (2.3%). También se evaluó la actividad antibacteriana del aceite frente a dos bacterias Gram (+) y dos Gram (-) encontrándose que el aceite presentó moderada actividad contra las bacterias Gram (+)

Palabras clave: Aceites esenciales, Lauraceae, *Ocotea caudata*, GC-MS, germacreno D

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INTRODUCTION

The Lauraceae is a family of about 2500 species distributed in the Neotropics of America and some species in Madagascar and Africa (van der Werff, 2002). This family is recognized by the economic importance, some species (particularly from the genus *Aniba*, *Nectandra* and *Ocotea*) have high commercial value because they are aromatic plants producing essential oils commonly used in industry (Marques, 2001). Essential oils can be found in roots, stems and fruits and the best known are the oils of rosewood, sassafras and cassia (Simić *et al.*, 2004). *Ocotea* is one of the largest genus of this family in America. In Colombia there are about 56 species of *Ocotea*. *Ocotea caudata* (Nees) Mez, is a tree whose geographic distribution in Colombia ranges from Pacific region to Orinoquia region. *O. caudata* is recognized traditionally by the names of “jigua”, “amarillo” o “laurel” (Klinger, 2009).

The phytochemical investigations on species of *Ocotea* shown this plants as a source of aporphine alkaloids, lignans, neolignans, phenylpropanoids and terpenes from essential oils (Chaverri *et al.*, 2011). Several essential oils of *Ocotea* have presented antioxidant, antibacterial, antifungal (Bruni *et al.*, 2004; Guerrini *et al.*, 2006), anti-inflammatory (Ballabeni *et al.*, 2010) and antiplatelet (Ballabeni *et al.*, 2007) activities. The present study reports the investigation of the antibacterial activity of essential oil from leaves of *Ocotea caudata*. This research is to the former, which to our knowledge, exist about studies of the oil essential of the leaves of *Ocotea caudata*.

MATERIAL AND METHODS

Plant material and essential oil isolation

Fresh leaves of *Ocotea caudata* were collected in August 2014, in the municipality of Puerto Lopez [coordinates: 4° 5' 28'' N and 73° 04' 21'' W], Meta Department, Colombia and the identity of the specimens was confirmed by biologist A. Jara. A voucher specimen was deposited at the Herbario Nacional Colombiano, Universidad Nacional de Colombia (COL544562). The fresh leaves (345 g) were subject to steam distillation for 2 h. The distilled oil were collected and dried over anhydrous sodium sulphate and stored in a freezer (0 - 5° C). The yield of the pale yellow oil from the leaf was 0.37% (w w⁻¹).

Essential oil analysis

The oil of *O. caudata* was analyzed by GC-MS using an Agilent Technologies 7890AGC gas chromatograph with split/splitless injector coupled to mass selective detector Hewlett Packard 5973, and two different systems of separation. The first was on a RTX-5MS column (60 m×0.25 mm×0.25 µm). Operating conditions were: carrier gas He, flow 1.0 mL min⁻¹; oven temperature program: 50° C (2 min) to 160° C (5 min) at 4° C min⁻¹, then rised to 220° C (5 min) at 2.5° C min⁻¹ and finally rised to 280° C (5 min) at 8° C min⁻¹; sample injection port temperature 250° C; detector temperature 285° C; ionization voltage: 70 eV; ionization current 60 µA; acquisition mass 35-400 m z⁻¹ range; split 1:20. The second was on a HP-INNOWax column (60 m×0.25 mm×0.25 µm) following the same operating conditions except the oven temperature program: 40° C (5 min) to 250° C (10 min) at 4° C min⁻¹. The compounds were identified by comparison of their retention indexes (RI) and mass spectra with those of NIST08, Wiley9L libraries spectra and the literature (Adams, 2007). Integration of the total chromatogram, expressed as area percent, has been used as a rough parameter for to ascertain the relative composition of the oil.

Antimicrobial activity

Antibacterial activity of the essential oil was determined by the agar well diffusion technique (Rios *et al.*, 1988). The microorganisms included two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6535) and *Bacillus subtilis* (ATCC 6638); two Gram-negative bacteria: *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027). The antibiotic Gentamicin (3 mg mL⁻¹) was used as positive control for the sensitivity of the tested bacteria. Data are expressed as mean ± S.D.

RESULTS AND DISCUSSION

The chemical composition of the leaves oil of *O. caudata* is summarized in Table 1. The identified components (37) represented 92.7% of all the components found in the oil. Sesquiterpene hydrocarbons were the major constituents (83.7%) of total oil and included germacrene D (55.8%), bicyclogermacrene (8.0%), β-caryophyllene (4.6%) and β-bourbonene (2.3%) as the main compounds.

The essential oil showed moderate activity only against Gram-positive bacteria (Table 2). The Minimal inhibitory concentration (MIC) of the

essential oil was 350 $\mu\text{g mL}^{-1}$ against *B. subtilis* and 500 $\mu\text{g mL}^{-1}$ against *S. aureus*.

Table 1
Main components of the essential oil of leaves of *Ocotea caudata*.

Compound	Approximate content in the Essential oil, %	RI
Hydrocarbons 0.1%		
Heptane	0.1	700
Monoterpene hydrocarbons 1.6%		
α -Pinene	0.2	940
Camphene	0.1	956
Sabinene	<i>tr</i>	979
β -Pinene	<i>tr</i>	985
β -Myrcene	<i>tr</i>	992
Limonene	0.1	1026
<i>cis</i> - β -Ocimene	1.2	1040
α -Terpinolene	<i>tr</i>	1091
Phenol 0.1%		
Thymol	0.1	1295
Sesquiterpene hydrocarbons 83.7%		
δ -Elemene	1.8	1347
α -Cubebene	0.1	1359
α -Ylangene	0.4	1383
β -Cubebene	1.2	1388
β -Bourbonene	2.3	1391
α -Gurjunene	0.6	1414
<i>cis</i>-β-Caryophyllene	4.6	1420
Calarene	0.3	1428
Aromadendrene	0.9	1432
<i>trans</i> - β -Caryophyllene	1.6	1437
α -Humulene	0.5	1464
γ -Muurolene	0.3	1482
γ -Amorphene	1.0	1487
Germacrene D	55.8	1490
(<i>trans, trans</i>)- α -Farnesene	1.6	1508
Bicyclogermacrene	8.0	1509
γ -Cadinene	0.4	1519
α -Amorphene	0.4	1527
δ -Cadinene	1.2	1530
Germacrene B	0.7	1565
Oxygenated Sesquiterpenes 7.2%		
Elemol	0.2	1557
<i>trans</i> -Nerolidol	1.1	1563
1 α ,10 α -epoxy-Amorph-4-ene	1.9	1583
Spathulenol	0.3	1585
Humulene epoxide II	0.4	1604
α -Cadinol	0.7	1653
α -Bisabolol	2.6	1692

RI: Retention index relative to n-alkanes C7-C24, compounds listed in order of elution in the RTX-5 column;
tr = traces (approximate content < 0.1%)

Table 2
Inhibition zone diameter (mm) of the essential oil of *Ocotea caudata* against four bacteria.

Oil Concentration, mg/mL	Diameter of inhibition (mm \pm S.D.)			
	<i>B.subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
5	8.0 \pm 0.0	14.1 \pm 0.0	NI	NI
10	12.1 \pm 0.0	18.9 \pm 0.0	NI	NI
20	15.6 \pm 0.3	21.8 \pm 0.3	NI	NI
Positive Control ^a	22.0 \pm 0.5	28.0 \pm 0.0	25.0 \pm 0.0	22.0 \pm 0.0

NI: No inhibition; ^a Gentamicine 1.5 mg mL⁻¹

The main component of the essential oil of *O. caudata* is germacrene D (55.8%), which is founded widely distributed at the plants, not only in angiosperms and gymnosperms but also in bryophytes, and its biological function in plants is still not well understood (Noge & Becerra, 2008).

Setzer (2008), summarize that essential oils containing high concentrations of germacrene D typically go with minor ratios of cadinane and muurolane sesquiterpenoids. Therefore has been proposed germacrene D as biogenetic precursor of some sesquiterpenoid skeletons such as cadinane, muurolane, and amorphane sesquiterpenes, when undergoes acid-catalyzed cyclization, and it is possible that these rearrangements may occur during obtaining the essential oil (Setzer, 2008; Noge & Becerra, 2008). This is evidenced at the essentials oil compositions. For instance, some species of *Ocotea* have shown large concentrations of germacrene D accompanied by smaller concentrations of γ -muurolene, α -muurolene, γ -cadinene, δ -cadinene, cadina-1,4-diene, and α -cadinene. (Setzer, 2008). In the case of the essential oil of *O. caudata* the ratios of these compounds are: germacrene D (55.8%), γ -muurolene (0.3%), γ -cadinene (0.4%), δ -cadinene (1.2%), α -amorphene (0.4%) and 1 α ,10 α -epoxy-Amorph-4-ene (1.9%). In terms of biological activity, germacrene D has deterrent effects against herbivores, insecticidal activity against mosquitoes, repellent activity against aphids and ticks (Noge & Becerra, 2008) and its known antimicrobial activity (De Lima et al., 2010)

On the other hand, investigations on essential oils of species of *Ocotea* show variability in their chemical compositions allowing classify it into two groups, the first related with terpene components and

the second with phenolic compounds (Lorenzo et al., 2001). The common terpene compounds to several species of *Ocotea*, either as major or trace components, are: α -pinene, β -pinene, β -elemene, β -caryophyllene, α -humulene, germacrene D, γ -cadinene, δ -cadinene and α -cadinol (Takaku et al., 2007). Moreover these nine compounds are common to other genera of the Lauraceae as *Beilschmiedia*, *Cinnamomum*, *Laurus*, *Lindera*, *Nectandra*, and *Persea* and they also were found in the essential oil of *O. caudata* which reveals the close chemotaxonomic relationship between *O. caudata* and other species of *Ocotea* belonging to the first group of chemical diversity (one whose species contain terpenes compounds) which is typical of essential oils of some species of *Ocotea* from South America (Chaverri et al., 2011). This work is the first report of the chemical composition and antibacterial activity of the essential oil of *O. caudata*.

The essential oil of *O. caudata* presented sesquiterpenes as the major constituents which could be considered as answerable for the antibacterial activity. Although the essential oil is a complex mixture of metabolites, their activity may generally account for in terms of their major components. Probably, germacrene D, bicyclogermacrene (De Lima et al., 2010) and β -caryophyllene could be responsible for this activity. β -caryophyllene has demonstrated be active against *S. aureus*, α -humulene, and elemol, have shown antimicrobial activity and α -bisabolol, seems to be responsible by antibacterial activity in *Lantana achyranthifolia* (Del-Vechio-Vieira et al., 2009; Kasim et al., 2014). However, in a mixture so complex, like the essential oil, it is difficult to attribute the activity to a single constituent. It is important consider that trace and

major components, as well as synergistic and antagonistic effect of compounds in the oil, might give rise to the antibacterial action described in the present investigation (Del-Vechio-Vieira *et al.*, 2009; Pirbaloutia *et al.*, 2013; Silvério *et al.*, 2013).

CONCLUSION

The study of the essential oil from *Ocotea caudata* yield the identification of 39 constituents (92.41% of the total oil) where germacrene D (55.82%), bicyclogermacrene (8.03%), β -caryophyllene (4.64%) and β -bourbonene (2.30%) were the main ones. The oil exhibited a moderated antibacterial activity against gram-positive bacteria.

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REFERENCES

- Adams RP. 2007. **Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry**. 4 th Ed, Allured, Carol Stream, IL, USA.
- Ballabeni V, Tognolini M, Bertoni S, Bruni R, Guerrini A, Moreno-Rueda G, Barocelli E. 2007. Antiplatelet and antithrombotic activities of essential oil from wild *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) calices from Amazonian Ecuador. **Pharmacol Res** 55: 23 - 30.
- Ballabeni V, Tognolini M, Giorio C, Bertoni S, Bruni R, Barocelli E. 2010. *Ocotea quixos* Lam. essential oil: *in vitro* and *in vivo* investigation on its anti-inflammatory properties. **Fitoterapia** 8: 289 - 295.
- Bruni R, Medici A, Andreotti E, Fantin C, Muzzoli M, Dehesa M, Romagnoli C, Sacchetti G. 2004. Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm.(Lauraceae) flower calices. **Food Chem** 85: 415 - 421.
- Chaverri C, Díaz C, Ciccio J. 2011. Chemical Analysis of Essential Oils from *Ocotea gomezii* W.C. Burger and *Ocotea morae* Gómez-Laur. (Lauraceae) Collected at “Reserva Biológica Alberto M. Brenes” in Costa Rica and their Cytotoxic Activity on Tumor Cell Lines. **J Braz Chem Soc** 22: 741 - 745.
- De Lima SG, Cito AMGL, Lopes JAD. 2010. Fixed and volatile constituents of genus *Croton* plants: *C. adenocalyx* baill – Euphorbiaceae. **Rev Latinoamer Quím** 38: 133 - 144.
- Del-Vechio-Vieira G, Sousa OV, Yamamoto CH, Kaplan MAC. 2009. Chemical Composition and Antimicrobial Activity of the Essential Oils of *Ageratum fastigiatum* (Asteraceae). **Rec Nat Prod** 3: 52 - 57.
- Guerrini A, Sacchetti G, Muzzoli M, Moreno-Rueda G, Medici A, Besco E, Bruni R. 2006. Composition of the volatile fraction of *Ocotea bofo* Kunth (Lauraceae) calyces by GC-MS and NMR fingerprinting and its antimicrobial and antioxidant activity. **J Agric Food Chem** 54: 7778 - 7778.
- Kasim LS, Olaleye KO, Fagbohun AB, Ibitoye SF, Adejumo OE. 2014. Chemical composition and antibacterial activity of essential oils from *Struchium sparganophora* Linn. Ktze Asteraceae. **Adv Biol Chem** 4: 246 - 252.
- Klinger W. 2009. Estado de conservación de las especies forestales amenazadas, abarco, jigua negro, guayaquil, guayacán amarillo y pino amarillo en los municipios chocoanos de Riosucio, Carmen del Darién, Istmina, Río Quito y Juradó. **Bioetnia** 6: 4 - 17.
- Lorenzo D, Loayza I, Leigue L, Frizzo C, Dellacassa E, Moyna P. 2001. Asaricin, the main component of *Ocotea opifera* Mart. essential oil. **Nat Prod Lett** 15: 163 - 170.
- Marques C. 2001. Importância econômica da família Lauraceae Lindl. **Floresta e Ambiente** 8: 195 - 206.
- Noge K, Becerra JX. 2009. Germacrene D, A Common Sesquiterpene in the Genus *Bursera* (Burseraceae) **Molecules** 14: 5289 - 5297.
- Pirbaloutia G, Firoznehada M, Crakerb L, Akbarzadehc L. 2013. Essential oil compositions, antibacterial and antioxidant activities of various populations of *Artemisia chamaemelifolia* at two phenological stages. **Rev Bras Farmacogn** 23: 861 - 869.
- Rios J, Recio M, Villar A. 1988. Screening methods

- for natural products with antimicrobial activity: A review of the literature. **J Ethnopharmacol** 23: 127 - 129.
- Setzer WN. 2008. Germacrene D Cyclization: An Ab Initio Investigation. **Int J Mol Sci** 9: 89 - 97.
- Silvério MS, Del-Vechio-Vieira G, Pinto MAO, Alves MS, Sousa OV. 2013. Chemical Composition and Biological Activities of Essential Oils of *Eremanthus erythropappus* (DC) McLeisch (Asteraceae) **Molecules** 18: 9785 - 9796.
- Simić A, Soković M, Ristić M, Grujić-Jovanović S, Vukojević J, Marin P. 2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. **Phytother Res** 18: 713 - 717.
- Takaku S, Haber W, Setzer W. 2007. Leaf essential oil composition of 10 Species of *Ocotea* (Lauraceae) from Monteverde, Costa Rica. **Biochem Syst Ecol** 35: 525 - 532.
- van der Werff H. 2002. A Synopsis of *Ocotea* (Lauraceae) in Central America and Southern Mexico. **Ann Missouri Bot Gard** 89: 429 - 451.