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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]

**Elizabeth Gil[1,2], Luis E. Cuca[1] & Wilman A. Delgado[1]**

[1] ***Laboratorio de Productos Naturales Vegetales, Departamento de Química. Universidad Nacional de Colombia, Bogotá DC, Colombia***
[2] ***Departamento de Química. Pontificia Universidad Javeriana, Bogotá DC, Colombia***
***Contactos | Contacts:*** **Elizabeth GIL-** ***E-mail address:*** **elgilar@unal.edu.co**

**Abstract:** *Ocotea* is a genus that belong to 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%), biciclogermacreno (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 sumarizes 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 μg $mL^{-1}$ against *B. subtilis* and 500 μg $mL^{-1}$ 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 | *tr* | 979 |
| β-Pinene | *tr* | 985 |
| β-Myrcene | *tr* | 992 |
| Limonene | 0.1 | 1026 |
| *cis*-β-Ocimene | 1.2 | 1040 |
| α-Terpinolene | *tr* | 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 |
| ***cis*-β-Caryophyllene** | **4.6** | 1420 |
| Calarene | 0.3 | 1428 |
| Aromadendrene | 0.9 | 1432 |
| *trans*-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 |
| (*trans*, *trans*)-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 |
| *trans*-Nerolidol | 1.1 | 1563 |
| 1α,10α-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 ± S.D.) | | | |
|---|---|---|---|---|
| | *B.subtilis* | S. aureus | E. coli | P. aeruginosa |
| 5 | 8.0 ± 0.0 | 14.1 ± 0.0 | NI | NI |
| 10 | 12.1 ± 0.0 | 18.9 ± 0.0 | NI | NI |
| 20 | 15.6 ± 0.3 | 21.8 ± 0.3 | NI | NI |
| Positive Control[a] | 22.0 ± 0.5 | 28.0 ± 0.0 | 25.0 ± 0.0 | 22.0 ± 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 distribuited at the plants, not only in angiosperms and gymnosperms but also in bryophites, 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 1α,10α-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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