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New alkaloids from *Ocotea duckei* vattimo (Lauraceae)

Novos alcaloides de *Ocotea duckei* vattimo (Lauraceae)

Nuevos alcaloides de *Ocotea duckei* vattimo (Lauraceae)

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Abstract
Ocotea duckei Vattimo, popularly known as “louro-de-cheiro”, “louro-pimenta”, and “louro-canela” is a member of the Lauraceae family found in the Northeast region of Brazil. It is popularly used to treat neuralgia, dyspepsia, anorexia and pain. This current study aimed to promote the isolation and the identification / determination of new secondary metabolites of the species *O. duckei* Vattimo. In order to obtain the vegetable drug of *O. duckei*, its stem barks were harvested and submitted to a drying process followed by pulverization. The Crude Ethanolic Extract (BSE), obtained from the vegetable drug, was then subjected to an alkaloid extraction protocol in order to generate its Total Alkaloid Fraction (TAF). The isolation and identification of chemical compounds were carried out by chromatographic and spectroscopic methods respectively. The chemical investigation of *O. duckei* resulted in the isolation of three compounds: Ocoteaduccin A (OD-1, 0.020 g), Ocoteaduccin B (OD-2, 0.015 g), and N-methylcoclaurine (OD-3, 0.008 g). *O. duckei* proved to be a promising species, due to the variety of isolated chemical compounds. Further chemical, pharmacological and toxicological studies must be conducted in order to improve the phytochemical knowledge about this plant.
Keywords: *Ocotea*; Lauraceae; Alkaloids; Chemical compounds.

Resumo
Ocotea duckei Vattimo, popularmente conhecido como “louro-de-cheiro”, “louro-pimenta” e “louro-canela” é um membro da família Lauraceae encontrada no Nordeste do Brasil. Popularmente é utilizado no tratamento de neuralgia, dispepsia, anorexia e dor. O presente estudo teve por objetivo promover o isolamento e a identificação/determinação de novos metabólitos secundários da espécie *O. duckei* Vattimo. A fim de obter a droga vegetal de *O. duckei*, as cascas do caule foram coletadas e submetidas ao processo de secagem. O Extrato Etanólico Bruto (EEB), obtido a partir da droga vegetal foi então direcionado a uma marcha para Alcaloides, resultando na Fração de Alcaloides Totais (FAT) correspondente. O isolamento e a identificação dos compostos químicos foram realizados por métodos cromatográficos e espectroscópicos respectivamente. O estudo fitoquímico de *O. duckei* resultou no isolamento de três substâncias: Ocoteaduccina A (OD-1, 0,020 g), Ocoteaduccina B (OD-2, 0,015 g) e N-metilcoclaurina (OD-3, 0,008 g). A *O. duckei* demonstrou ser uma espécie promissora, pela variedade de compostos químicos isolados. Mais

estudos químicos, farmacológicos e toxicológicos devem ser feitos a fim de melhorar o conhecimento fitoquímico acerca desta planta.
Palavras-chave: *Ocotea*; Lauraceae; Alcaloides; Compostos químicos.

Resumen
Ocotea duckei Vattimo, conocida popularmente como "laurel-olfato", "laurel-pimienta" y "laurel-canela" es un miembro de la familia Lauraceae que se encuentra en el noreste de Brasil. Se utiliza popularmente para tratar la neuralgia, la dispepsia, la anorexia y el dolor. El presente estudio tuvo como objetivo promover el aislamiento y la identificación / determinación de nuevos metabolitos secundarios de la especie *O. duckei* Vattimo. La corteza del tallo después de la recolección, pasó por el proceso de secado y fue triturada para obtener la droga vegetal. El Extracto Etanólico Crudo (EEB) se obtuvo a partir de la droga vegetal, de la EEB se realizó una marcha a Alcaloides, obteniendo así la Fracción de Alcaloides Totales (FAT). El aislamiento y la identificación de compuestos químicos se llevaron a cabo mediante métodos cromatográficos y espectroscópicos respectivamente. El estudio fitoquímico de *O. duckei* dio como resultado el aislamiento de tres sustancias: ocoteaduccina A (OD-1, 0,020 g), ocoteaduccina B (OD-2, 0,015 g), N-metilcoclaurina (OD-3, 0,008 g). *O. duckei* resultó ser una especie prometedora, debido a la variedad de compuestos químicos aislados. Se deben realizar más estudios químicos, farmacológicos y toxicológicos para promover el conocimiento sobre esta planta.
Palabras clave: *Ocotea*; Lauraceae; Alcaloides; Compuestos químicos.

1. Introduction

Brazil has a very diversified flora, represented by a vegetation of different characteristics and many active ingredients that are still unknown, which justifies the significant increase of studies related to products of plant origin aiming at obtaining new potential herbal medicines (Martins, 2018).

Due to the structural chemical diversity of bioactive compounds obtained from plants, research is conducted to better understand the clinical applicability of substances derived from natural products (Oliveira, 2018).

The Lauraceae family consists of about 50 genera and 3000 species, being the fourth largest family concerning the number of species (Beech et al., 2017). The family is chemically characterized by the presence of mainly isoquinoline and indole alkaloids (Barbosa-Filho; Yoshida; Gottlieb, 1989), lignans and neolignans (Gottlieb; Yoshida, 1989), and essential oils, generally composed of monoterpenes, sesquiterpenes and phenylprapranoids (Pino et al., 2005).

The largest genus of Lauraceae is *Ocotea*, with 428 species (The Plant List 2013), widely distributed through tropical and subtropical America, from Mexico to Argentina, considered one of the most representatives of the family in the Atlantic Forest (Oliveira-Filho & Fontes 2000, Santos & Alves 2012). In Brazil, the genus is present throughout the national territory, with 172 species, among which, 49 are found in the state of São Paulo (Quinet et al. 2015).

Ocotea duckei Vattimo, popularly known as “louro-de-cheiro”, “louro-pimenta”, and “louro-canela” (Barreto, 1990) is a member of the Lauraceae family found in the Northeast of Brazil. It is popularly used to treat neuralgia, dyspepsia, anorexia and pain (Villamizar, 2010).

Regarding previous chemical investigation of *O. duckei* species, several lignans were reported, such as: yangambine, epiyangambine, sesartemine, episesartemine, syringaresinol, 4'- O- dimethyl epimagnolin A, and (+) - 4 " - O- dimethyl epimagnolin A (Morais et al., 1996; Morais et al., 1998a). Three benzylisoquinoline alkaloids were isolated: reticulin (Morais et al., 1998b), coclaurine (Da Silva et al., 2002), and N-acetylnorjuzifine (Dias et al., 2003). An aporphine alkaloid, laureliptin, was also reported (Dias et al., 2003).

The analysis of essential oils extracted from different parts of *O. duckei* has demonstrated that they were constituted of complex mixtures of monoterpenes and sesquiterpenes, with trans-karyophylene representing its major component, followed by α-humulene and δ-selinene sesquiterpenes, which also represented a significant percentage of the essential oil. In relation to the chemical composition of the essential oil from its fruits, high concentration of d-limonene was detected. As for the stem, it was detected a predominance of β-eudesmol, whereas in the root, the main constituent was elemol (Lacerda, 2004).

Thus, considering the chemical-pharmacological potential of plant species found in Paraiba biomes, especially from the Lauraceae family, this work aims to continue the chemical investigation of *O. duckei* Vattimo, through the use of chromatographic techniques, by analyzing the chromatographic profile of each corresponding fractions, to finally isolate and purify its constituents.

2. Methodology

2.1 Harvesting and identification of botanical material

The stem bark of *O. duckei* was collected in the city of Santa Rita, state of Paraíba. The botanical material was identified by Professor Dr. Maria de Fátima Agra, from the Biotechnology Center (Cbiotec / UFPB) and a voucher specimen was deposited in Prof. Lauro Pires Xavier (JPB) Herbarium, located in the Center for Exact Sciences and Nature (CCEN / UFPB) under the reference of AGRA 4309. This species was also registered under code number A8EE18F, in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen-Brasil).

2.2 Extraction and fractionation

The plant material was oven-dried for 72 hours at 40 ºC with the aid of an air circulating system. After drying, the botanical material was subjected to a grinding process in a mechanical mill, resulting in 6,500 g of plant powder, which was subjected to a maceration process with 80% ethanol at room temperature for 4 days. The corresponding extractive solution was concentrated in a rotary evaporator under reduced pressure, at an average temperature of 40 ° C, to finally obtain 600 g of crude ethanolic extract (CEE).

The CEE was suspended in a 3% solution of HCl, filtered over Celite and extracted several times with $CHCl_3$. The aqueous fraction was basified with NH_4OH up to pH 9 and extracted again with CHCl3. The CHCl3 extract was washed with H_2O, dried with Na_2SO_4 and concentrated under reduce pressure to obtain a Total Alkaloid Fraction (TAF) of 9.2g.

2.3 TAF fractionation

An aliquot of the TAF was subjected to column chromatography (CC) using aluminum oxide as stationary phase (oxide 90, activity II-III, particle size 0.063-0.200 mm, MERCK) and $CHCl_3$ and MeOH as mobile phase, which were used as binary mixtures with an increasing degree of polarity in order to obtain three fractions. These three fractions were submitted to preparative thin layer chromatography (PTLC), which were eluted with $CHCl_3$: MeOH (6.5: 3.5). The PTLC fractionation has enabled the identification of the three compounds by the combination of several spectroscopic techniques. Optical rotations analyses were measured in MeOH (ADP 220, Bellingham + Stanley Ltd), IR spectra (FT-IR spectrometer, model MB 100M, BOMEM) were recorded in KBr pellets, and NMR spectral analyses (VARIAN MERCURY 200 and BRUKER AC 500) were performed on CD_3OD and $CDCl_3$. Thus identifying the three substances: Ocoteaduccin A (OD-1, 0.020 g), Ocoteaduccin B (OD-2, 0.015 g), N-methylcoclaurine (OD-3, 0.008 g).

3. Results and Discussion

Compound **(OD-1)** was isolated with an oily aspect. Mass spectrum showed an $[M]^+$ *m/z* 385, corresponding to the proposed formula $C_{23}H_{31}NO_4$. The NMR study (^{1}H, ^{13}C-COSY-$^1J_{CH}$, ^{1}H-^{13}C-COSY-$^nJ_{CH}$, ^{1}H-^{1}H-COSY and ^{1}H-^{1}H-NOESY, optimized for J=7 Hz), led to unambiguous assignment of all functional groups. The ^{1}H NMR (500 MHz, $CDCl_3$) spectrum showed two singlets at δ 6.70 and 6.12 and an AA'BB' system at δ 6.94 and 6.71 (J_{AB}=8.4 Hz) in the aromatic region, characteristic for 6, 7, 12 trioxygenated tetrahydrobenzylisoquinoline[10]. Other significant signals included two singlet at δ 1.24

for 3H ($2CH_3$), two multiplets at δ 3.59 (H-1') and 2.67 (H-2') for 2H ($2CH_2$), supported by a quaternary C signal at δ 70.0 ppm (APT, DEPT), and the signals at δ 57.0 and 64.0 ($-CH_2-CH_2-$) ppm. The ^{13}C NMR spectrum confirmed the presence of a prenyl substituent. The relatively lowfield shift at δ 57.0 ppm for C2' ($-CH_2-$) suggested a β deproctetion effect ($2CH_3$ and OH attached to the C3'). Other signals at δ 3.80 (MeO) and δ 2.66 (NMe) were observed. The location of the MeO group at C-6 was assigned by HMBC spectrum from their ^{13}C-^{1}H long range coupling between δ_H 3.80 ($C\underline{H_3}O$-) and δ_C 148.8 (C6).

Compound **(OD-2)** was obtained with an oily aspect. Its mass spectrum showed an $[M]^+$ *m/z* 415, corresponding to the formula $C_{24}H_{33}NO_5$. It differs of **1** by showing in the ^{1}H NMR spectrum one singlet at δ 3.83 integrating for three hydrogens and ABX system at δ 6.67 (H-10), δ 6.86 (H-13), δ 6.60 (H-14), (J_{ABX}= 8.2 and 1.8 Hz) indicated that ring C was trisubstituted. HMBC showed correlations between the signals at δ_H 3.38 (CH_3O-12) and δ_C 148.8 (C-12) consistent with the OCH_3 attached to that position. The analysis of the ^{13}C NMR spectrum of **1** and **2** showed signals for 23 and 24 carbons confirming the assigned molecular formula. Complete assignment of all protons and carbon atoms are given above.

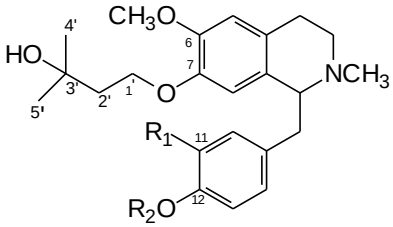
Figure 1 shows the isolated structures of *Ocotea duckei.*

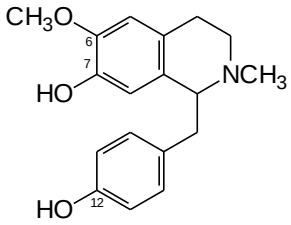
Ocoteaduckeine A (OD-1): viscous oil, $[\alpha]_D^{25}$ = + 50 (*c*, 0,01, MeOH). IR (KBr) ν_{max} (cm^{-1}): 2926, 2851, 1613, 1515, 1448, 1374, 1172, 1110, 1019, 871, 833, 801, 547. UV λ_{max} (nm): 226, 236, 281, 284. ^{1}H NMR δ (ppm): 6.94 (2H, *d*, *J*=8.4 Hz, H-10 and H-14), 6.71 (2H, *d*, *J*=8.4 Hz, H-11 and H-13), 6.70 (1H, *s*, H-5), 6.12 (1H, *s*, H-8), 4.03 (1H, *dd*, *J*=13.0 and 5.5 Hz, H-1), 3.81 (3H, *s*, CH_3O-6), 3,59 (2H, *m*, H-1'), 3.38 and 2.98 (2H, *m*, H-3), 3.15 (1H, *dd*, *J*=13.0 and 5.5 Hz, H-15a) and 2.88 (1H, *dd*, *J*=13.0 and 7.8 Hz, H-15b), 2.94 and 2.82 (2H, *m*, H-4), 2.67 (2H, *m*, H-2'), 2.66 (3H, s, N-CH_3), 1.24 (3H, *s*, H-4'), 1.24 (3H, s, H-5'). ^{13}C NMR δ (ppm): 157.5 (C-12), 148.8 (C-6), 145.9 (C-7), 131.9 (C-10 and C-14), 129.6 (C-9), 127.6 (C-8a), 124.0 (C-4a), 116.5 (C -11 and C-13), 115.9 (C-8), 112.7 (C-5), 70.0 (C-3'), 66.2 (C-1), 64.5 (C-1'), 57.0 (C-2'), 56.3 (CH_3O-6), 47.4 (C-3), 41.8 (CH_3-N), 40.7 (C-15), 29.7 (C-4' and C-5'), 24.9 (C-4).

Ocoteaduckeine B (OD-2): viscous oil**,** $[\alpha]_D^{25}$ = + 40 (*c*, 0,025, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3418, 2925, 2852, 2361, 1513, 1440, 1376, 1276, 1131, 1108, 1021, 871, 761. UV λ_{max} (nm): 230, 236, 283, 289. ^{1}H NMR δ (ppm): 6.86 (1H, *d*, *J*=8.2 Hz, H-13), 6.75 (1H, *s*, H-5), 6.67 (1H, *sl*, H-10), 6.60 (1H, *dl*, *J*=8.2 Hz, H-14), 6.21 (1H, *s*, H-8), 4.28 (1H, ***t***, *J*=6.0, Hz, H-1), 3.84 (3H, *s*, CH_3O-6), 3.83 (3H, *s*, CH_3O-12), 3.59 (2H, *m*, H-1'), 3.50 and 3.20 (2H, *m*, H-3), 3.17 (1H, *dd*, *J*=14.0 and 5.2 Hz, H-15a) and 2.98 (1H, *m*, H-15b), 3.02 and 2.97 (2H, *m*, H-4), 2.80 (3H, *s*, N-CH_3), 2.67 (2H, *m*, H-2'), 1.26 (3H, *s*, H-4'), 1.26 (3H, *s*, H-5'). ^{13}C NMR δ (ppm): 149.2 (C-6), 148.8 (C-12), 148.5 (C-11), 146.4 (C-7), 130.5 (C-9),125.7 (C-8a), 122.9 (C-4a), 122.1 (C -14), 117.5 (C-10), 115.5 (C-8), 112.7 (C-13), 70.9 (C-3'), 66.2 (C-1), 64.5 (C-1'), 57.2 (C-2'), 56.6 (CH_3O-12), 56.5 ($C\underline{H_3}O$-6), 47.3 (C-3), 41.4 ($C\underline{H_3}N$-2), 40.8 (C-15), 29.7 (C-4'). 29.7 (C-5'), 24.1 (C-4).

***N*-Metylcoclaurine (OD-3)**: brownish amorphous power, $[\alpha]_D^{23}$ = + 64 (*c*, 0,1, MeOH)[7]. IR (KBr) ν_{max} (cm^{-1}): 3600 and 2850. UV (MeOH) λ_{max} (log ε): 288 (3,6) and 231 (4.1) [8,9] . ^{1}H NMR δ (ppm): 6.95 (2H, *d*, *J*=8,6, H-10 and H-14), 6.67 (2H, *d*, *J*=8.6 Hz, H-11 and H-13), 6.66 (1H, *s*, H-8), 6.48 (1H, *s*, H-5), 3.7 (3H, s, CH_3O-6), 2.32 (3H, *s*, CH_3N-2). ^{13}C NMR δ (ppm): 155,4 (C-12), 145.8 (C-6), 143.8 C-7), 130.1 (C-10 and C-14), 129,4 (C-9), 128.6 (C-8 a), 125.3 (C-4 a), 115.4 (C-13), 112.4 (C-8), 111.2 (C-5), 64.0 (C-1), 55.5 (CH_3O-6), 45.9 (C-3), 41.7 (CH_3N-2), 40.0 (C-15), 24.4 (C-4).

Figure 1. Molecules isolated from *Ocotea duckei*.





1. $R_1 = R_2 = H$ **3**

2. $R_1 = OH$, $R_2 = CH_3$

Source: Authors.

4. Conclusion

The current work has contributed to expand the phytochemical knowledge of the genus *Ocotea*, through the chemical investigation of the species *O. duckei*, represented by the isolation three alkaloids (OD-1; OD-2 and OD-3), as well as its identification by fundamental spectroscopic techniques such as ^{1}H and ^{13}C Nuclear Magnetic Resonance (1D and 2D).

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