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Contribution to the phytochemical study of *Ocotea gardneri* (MEISN) MEZ (Lauraceae).

MELO,G.I.G.[1]; PEIXOTO,L.R.[2]; TELES,M.M.S.[3]; LIMA,N.R.[4]; SOUZA,G.[5]; FIGUEIREDO, F.[6]; FILHO,J.M [7]

[1] *Federal University of Paraiba*
[2] *Federal University of Paraiba*
[3] *Federal University of Paraiba*
[4] *Federal University of Paraiba*
[5] *Federal University of Paraiba*
[6] *Federal University of Paraiba*
[7] *Federal University of Paraiba*

Graphical Abstract	**Abstract.**
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Introduction *(optional)*

Most of the drugs in clinical use are of natural origin or were developed by chemical synthesis planned from natural products. Although there are several strategies and methodologies available today to synthesize and discover new drugs, the chemistry of natural products represents one of these historically privileged success alternatives (BARREIRO; SILVA-BOLZANI, 2009).

The Lauraceae family, belonging to the order Laurales, is considered one of the most primitive families of the Magnoliphyta division (STEVENS, 2016). It was established by Antoine Laurent de Jussieu in 1789 and the knowledge of its species dates from 2800 BCE, with reports of the use of Cinnamomum camphora (L.) J.Presl oil and other species of the genus in Chinese medicine. The leaves of laurus nobilis L., were used as wreaths by the ancient Greeks and Romans to crown warriors and athletes (GOTTLIEF, 1972).

The genus *Ocotea* presents about 350 species, most in the tropical and subtropical Americas. It is estimated that Brazil hosts approximately 160 plant species of this genus (QUINET et al., 2016). Their species do not have constancy in the fruiting, fact that hinders their propagation (ZANIN, LORDELLO, 2007). The genus *Ocotea* arouses the phytochemical interest due to its distribution throughout the national territory and by the numerous pharmacological activities attributed to the lignans and alkaloids present in its species (SOUZA et al., 2004).

Ocotea gardneri (Meisn) Mez is a species found in the Brazilian Northeast, especially in the states of Paraíba, Pernambuco. It is known as "blonde baboon" or "white blonde" (BARRETO, 1990). Quinet and Andreata (2002) describe this species as being a synonym of *Ocotea nonata* Mez, and Rohwer described it as such. According to these authors, these species are differentiated by the shape of the leaves and mainly by the type of habitat.

Materials and Methods *(optional)*

The plant material (leaves) of *Ocotea gardneri* was collected in the municipality of Santa Rita, in February 2012, state of Paraíba. The aerial parts of the plant species were dehydrated in an oven. It was subjected to maceration with 95% ethanol for 72 hours, the solutions were concentrated in a rotary evaporator, obtaining the Brine Ethanol Extract (EEB) from *Ocotea gardneri*. EEB was partitioned, used a methanol / water (7: 3) mixture, and the hydrazoethanol solution was obtained which was subjected to a separatory funnel with hexane, dichloromethane and ethyl acetate. The phases were then dehydrated with anhydrous sodium sulfate, filtered and concentrated on a rotary evaporator under reduced pressure.

The dichloromethane phase of the BSE was subjected to column chromatography (CC), using as silica gel 60 stationary phase and as solvent phase hexane, dichloromethane and methanol, pure or in binary mixtures, the fractions being concentrated in a rotary evaporator. The fractions were analyzed by analytical thin layer chromatography using different elution systems and pooled, when similar, after visualization in ultraviolet light and impregnation with iodine vapors were collected in 11 groups. Fraction 17-21 (73.0 mg) after CCDA analysis revealed a single spot and was then subjected to ^{1}H and ^{13}C NMR spectroscopy and encoded as Og-1.

Fraction 60-73 (200.0 mg) was subjected to CC using as the flash silica stationary phase and as eluents pure hexane, dichloromethane and methanol or in binary mixtures with increasing polarity gradient. Twenty fractions were obtained. Subpraction 7-10 was subjected to ^{1}H and ^{13}C NMR spectroscopy, encoded as Og-2 (25 mg). Fraction 90-116 (30.0 mg) after CCDA analysis revealed a single spot, undergoing ^{1}H and ^{13}C NMR spectroscopy, and then encoded as Og-3.

The ethyl acetate phase was subjected to CLMP (liquid medium pressure chromatography) using the Büchi® chromatograph and as the mobile phase pure dichloromethane, ethyl acetate and methanol solvents or in binary mixtures, obtaining There were 47 fractions of 50 mL each. The fractions were concentrated on rotary evaporator under reduced pressure. Then, the fractions were analyzed by CCDA and assembled according to their chromatographic profiles. Fraction 4-7 (200 mg) was subjected to CC using sephadex-LH20 as fixed phase and methanol as the mobile phase, yielding 14 subfractions which were pooled into 5 groups after CCDA monitoring. From this chromatographic procedure a yellow powder was obtained which after ^{1}H and ^{13}C NMR analysis, the substance was encoded as Og-4 (43 mg).

Fraction 19-29 (350mg) was also submitted to CC similar to the above process, with 22 subfractions being collected in 5 groups after CCDA monitoring. Fraction 8-15 (150 mg), in turn, was submitted to the new CC using a reverse phase C18 cartridge and as eluent methanol, obtaining 7 subfractions of 10 mL each, which were analyzed by CCDA. Fractions 3 and 4 of subfraction 10 after CCDA analysis were encoded as Og-5 (39 mg) and Og-6 (27 mg).

Results and Discussion *(optional)*

A substance encoded as Og-1 was completed as a colorless oil with 73 mg. The ^{13}C-APT NMR spectrum (50 MHz, $CDCl_3$) showed signals in δC 25,7 (C-1 e C-24), δC 131,2 (C-2 e C-23), δC 124,3 (C-3 e C22), δC 26,5 (C-4 e C-21), δC 39,7 (C-5 e C-20), δC 135,0 (C-6 e C-19), δC 124,3 (C-7 e C-

18), δC 28,3 (C-8 e C-17), δC 39,7 (C-9 e C-16), δC 134,8 (C-10 e C-15), δC 124,3 (C-11 e C-14), δC 20,7 (C-12 e C-13), δC 17,7 (C-25 e C-30), δC 16,0 (C-26 e C-29), δC 16,0 (C-27 e C-28). The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of Og-1 showed signals in δH 5,12 (t, J = 4 Hz, H3, H-7, H-11, H-14, H-18 e H-22), δH 2,01 (q, J = 6,0 Hz) attributed to the methylene hydrogens neighboring double bonds, δH 1,60 (s, H-25, H-26, H-27, H-28, H-29 e H-30), δH 1,68 (s, H-1 e H-24). The NMR data, compared with the literature data, allowed us to infer that Og-1 was 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexene, known such as squalene.

The substance encoded as Og-2 was obtained as a white amorphous solid of 25 mg. The ^{1}H-NMR spectrum (200 MHz, pyridine-d_5) showed signals in δH 7,31 (s, 2H, H-2), δH 7,31(s, 2H, H-6), δH 10,02 (s, 1H, O=CH), δH 3,90 (s, 3H, *p*-OCH_3), δH 3,75 (s, 6H, *m*-OCH_3). In the ^{13}C-APT NMR spectrum (50 MHz, pyridine-d_5), showed signals in δC 132,9 (C-1), δC 107,5 (C-2), δC 154,6 (C-3), δC 144,4 (C-4), δC 154,6 (C-5), δC 107,6 (C-6), δC 191,8 (O=CH), δC 61,0 (*p*-OCH_3), δC 56,4 (*m*-OCH_3). The signs shown corroborate 3,4,5-Trimethoxybenzaldehyde, first reported in *Ocotea Gardner*.

The substance encoded as Og-3 was isolated as white crystals with 30 mg of melting point 292-294 ° C. The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) showed signals in δH 3,49 (*m*, 1H, H-1), δH 5,32 (d, J = 5,0 Hz, 1H, H-6), δH 0,66 (s, 3H, H-18), δH 0,98 (s, 3H, H-19), δH 0,89 (d, J = 6,4 Hz, 3H, H-26), δH 0,79 (d, J = 5,6 Hz, 3H, H-27). In the ^{13}C-APT NMR spectrum (125 MHz, $CDCl_3$) showed signals in δC 37,60 (C-1), δC 30,30 (C-2), δC 78,30 (C-3), δC 39,40 (C-4), δC 141 (C-5), δC 122 (C-6), 32,20 (C-7), 32,10 (C-8), δC 50,40 (C-9), δC 37 (C-10), δC 21,40 (C-11), δC 40 (C-12), δC 42,4 (C-13), δC 56,3 (C-14), δC 24,6 (C-15), δC 28,7 (C-16), δC 56,5 (C-17), δC 12 (C-18), δC 19,3 (C-19), δC 36,5 (C-20), δC 19,1 (C-21), δC 34,3 (C-22), δC 26,4 (C-23), δC 46,1 (C-24), δC 29,5 (C-25), δC 20,1 (C-26), δC 19.5 (C-27), δC 23,4 (C-28), δC 12,2 (C-29). The signals identified identified 0g-3 as Sitosterol-3-O-β-D-glucopyranoside, reported for the first time in *Ocotea gardneri*.

The substance encoded as Og-4 was obtained as a yellow powder with 43 mg and melting point 270 272 ° C. The ^{13}C -NMR spectrum (50 MHz, in CD_3COCD_3): δC 145,7 (C-2), δC 136,7 (C-3), δC 176,5 (C-4), δC 162,3 (C-5), δC 99 (C-6), δC 164,9 (C-7), δC 94,4 (C-8), δC 157,7 (C-9), δC 105,9 (C-10), δC 123,7 (C-1'), δC 115,7 (C-2'), δC 148,2 (C-3'), δC 145,7 (C-4'), δC 116,1 (C-5'), δC 121,4 (C-6'). The ^{1}H-NMR spectrum (200 MHz, in CD_3COCD_3): δ_H 6,262 (d, J = 1,8 Hz, 1H, H-6), δ_H 6,522 (d, J = 1,8 Hz, 1H, H-8), δ_H 7,822 (d, J = 2,0 Hz, 1H, H-2'), δ_H 6,993 (d, J = 8,4 Hz, 1H, H-6'), δ_H 7,695 (dd, J = 8,6; 2,0 Hz, 1H, H-6'). It was identified as 3,5,7,3 ', 4'-pentahydroxyflavone.

The substance encoded as Og-5 was obtained as colorless crystals of 39 mg and mp 175-177 ° C. NMR spectrum analysis of ^{1}H (200 MHz, in CD_3OD): δ_H 4,564 (d, J = 7.5 Hz, 1H, H-2), δ_H 3,92-4,0 (m, 1H, H-3), δ_H 2,852 (dd, J = 16,0, 5,4 Hz, 1H, H-4a), δ_H 2,50 (dd, J = 16,1, 8,0 Hz, 1H, H-4b), δ_H 5,929 (d, J = 2,4 Hz, 1H, H-6), δ_H 5,854 (d, J = 2,4 Hz, 1H, H-8), δ_H 6,837 (d, J = 1,4 Hz, 1H, H-2'), δ_H 6,750 – 6,728 (m, 2H, H-5'), δ_H 6,750 – 6,728 (m, 2H, H-6'). In the ^{13}C-NMR spectrum (50 MHz, CD_3OD): δ_C 82,8 (C-2), δ_C 68,8 (C-3), δ_C 28,5 (C-4a), δ_C 157,6 (C-5), δ_C 96,2 (C-6), δ_C 157,8 (C-7), δ_C 95,5 (C-8), δ_C 156,9 (C-9), δ_C 100,8 (C-10), δ_C 132,2 (C-2'), δ_C 115,2 (C-2'), δ_C 146,2 (C-3'), δ_C 146,2 (C-4'), δ_C 116 (C-5'), δ_C 120 (C-6'). Then we can identify as Og-5 as the catechin.

The substance encoded as Og-6 was obtained as colorless crystals with 27 mg and melting point 178-180°C. NMR spectrum analysis of ^{1}H (200 MHz, CD_3OD): δ_H 4,803 (s, 1H, H-2), δ_H 4,165 (s, 1H, H-3), δ_H 2,862 (dd, J = 16.8 e 4,4 Hz, 2H, H-4a), δ_H 2,715 (dd, J = 16, 8 e 2,8 Hz, 1H, H-4b), δ_H 5,928 (d, J = 2.4 Hz, 1H, H-6), δ_H 5,905 (d, J = 2,2 Hz, 1H, H-8), δ_H 6,964 (d, J= 1,6 Hz, 1H, H-2'), δ_H 6,777 – 6,761 (m, 2H, H-5'), δ_H 6,777 – 6,761 (m, 2H, H-6'). ^{13}C-NMR (50 MHz, CD_3OD) spectral data: δ_C 79,8 (C-2), δ_C 67,5 (C-3), δ_C 29,3 (C-4a), δ_C 158 (C-5), δ_C 96,3 (C-6), δ_C 157,3 (C-7), δ_C 95,9 (C-8), δ_C

157,6 (C-9), δ_C 100 (C-10), δ_C 132,3 (C-1'), δ_C 115,3 (C-2'), δ_C 145,7 (C-3'), δ_C 145,9 (C-4'), δ_C 115,9 (C-5'), δ_C 119,4 (C-6'). We identified as Og-6 epicatechin.

Conclusions *(optional)*

The phytochemical study of the aerial parts of *Ocotea gadneri* resulted in the isolation and structural identification of six substances unknown to the species. Three substances were isolated from the chloroform phase, triterpene 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexene (squalene), 3,4,5-trimethoxybenzaldehyde and Sitosterol-3-O-β-D-glucopyranoside. From the ethyl acetate phase, flavone 3,5,7,3 ', 4'-pentahydroxyflavone (quercetin) and two flavonols 2- (3,4-dihydroxyphenyl) -4H-chromene-3,5,7- triol (catechin) and 2- (3,4-dihydroxyphenyl) -4H-chromene-3,5,7-triol (epicatechin).

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