

Artículo Original / Original Article

***Ocotea* (Lauraceae) Amazonian essential oils chemical composition and their tyrosinase inhibition to use in cosmetics**

[*Ocotea* (Lauraceae) composición química de aceites esenciales amazónicos y su inhibición de la tirosinasa para usar en cosméticos]

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Abstract: This study presents analyses on the chemistry, biology, pharmacology and chromatography of essential oils extracted from three species of the *Ocotea* genus: *O. minor*, *O. ceanothifolia* and *O. leucoxylon*. Leaves and stems, as well as the bark of *O. minor*, were processed using a modified Clevenger apparatus. Seven essential oils were obtained and analyzed using GC-FID and GC-MS, and their chemical compositions were determined. Assays of cytotoxicity, antioxidant and free radical scavenging activity, as well as tyrosinase and elastase inhibition were performed. In total, 25 constituents were identified, the principal being sesquiterpenes, such as spathulenol caryophyllene and its oxide. The oils did not present cytotoxicity using a hemolytic model, but also did not show antioxidant activity in the DPPH assay. Essential oil from stems of *O. ceanothifolia*, rich in spathulenol and caryophyllene oxide, demonstrated the capacity to inhibit 49.08% of tyrosinase activity at a concentration of 100 µg/mL. This research contributes to the chemical profile analysis of the three species of *Ocotea* through chemical investigations and biological activity, which are reported for the first time here in this study.

Keywords: Amazon oil; GC-MS; Lauraceae; Tyrosinase inhibition; Caryophyllene, *Ocotea* genus

Resumen: Este trabajo realiza un estudio químico, biológico, farmacológico y cromatográfico de aceites esenciales extraídos de tres especies del género *Ocotea*: *O. minor*, *O. ceanothifolia* y *O. leucoxylon*. Las hojas y tallos, así como la corteza de *O. minor*, se procesaron utilizando un aparato Clevenger modificado. Se obtuvieron siete aceites esenciales y se analizaron usando GC-FID y GC-MS, y se determinaron sus composiciones químicas. Se realizaron ensayos de citotoxicidad, actividad antioxidante y de atrapamiento de radicales libres, así como inhibición de tirosinasa y elastasa. En total, se identificaron 25 componentes, siendo los principales sesquiterpenos, como el spathulenol cariofileno y su óxido. Los aceites no presentaron citotoxicidad en un modelo hemolítico y tampoco mostraron actividad antioxidante en el ensayo con DPPH. El aceite esencial de tallos de *O. ceanothifolia*, rico en espatulenol y óxido de cariofileno, mostró capacidad para inhibir el 49.08% de la actividad de tirosinasa a una concentración de 100 µg/mL. Esta investigación contribuye al análisis del perfil químico de las tres especies de *Ocotea* a través de investigaciones químicas y actividad biológica la cual se informan por primera vez.

Palabras clave: Aceite de Amazonas; GC-MS; Lauraceae; Inhibición de la tirosinasa; Cariofileno; Género *Ocotea*

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INTRODUCTION

The skin is the largest organ of the human body and protecting it is important to minimize the effects of aging. The worldwide production of cosmetics with anti-aging capabilities is growing and the use of natural actives has been highlighted for the benefits they offer (Mellou *et al.*, 2019). Essential oils, in particular, have been used as fixing agents in perfumes, antimicrobial preservatives and recently, research has shown their potential for use in skin cosmetics (Yamaguchi *et al.*, 2013; Carvalho *et al.*, 2016).

Lauraceae is an important family, with 2500 species distributed in the American Neotropics and some species in parts of Africa (Van Der Werff, 2013). Species of this family are known for producing essential oils that are used in both traditional medicine and industry (Marques, 2001). From this family, *Aniba rosaeodora* (rosewood) oil is widely used in perfume fragrances and its main constituent is the terpenic alcohol linalool with a mixture of D- and L-linalool isomers, which varies according to where the sample was collected. Similarly, camphor extracted from *Cinnamomum camphora*, and safrole extracted from *Ocotea pretiosa* and *Sassafras albidum*, are also commonly used in the cosmetic industry as a scent in perfumes and soaps (Rizzini & Mors, 1995).

Among the genera of this family, *Ocotea* Aublet is the largest in the neotropical region and presents the highest number of species with medicinal properties, as both its extracts and essential oils are commonly used for therapeutic purposes (Marques, 2001). This genus consists of around 350 species, distributed throughout tropical and subtropical America, from México to Argentina, occurring also in the Canary Islands and parts of Africa such as Madagascar (Rhower, 1993).

Oils from species of *Ocotea* found in many Latin American countries are commonly described in the literature as containing in their chemical composition: monoterpenes such as pinene, linalool and limonene; sesquiterpenes such as germacrene, humulene, caryophyllene and its oxide; phenylpropanoids like safrole and methyl eugenol; and derivatives of cinnamaldehyde and benzenoids (Gottlieb *et al.*, 1981; Ballabeni *et al.*, 2010; Chaverri *et al.*, 2011; Yamaguchi *et al.*, 2013; Gil *et al.*, 2016).

When investigating applications of natural extracts in dermocosmetics, activities evaluated typically include free radical scavenging capacity to assess antioxidant activity, which can contribute to

cell preservation, tyrosinase inhibition, which can act as a dermatological treatment for hyperpigmentation, and elastase inhibition, which assists in the management of the physical properties of the skin. The results of these tests are important for the appropriate use of the final product, since following its cytotoxic capacity is essential for its overall safety (Teixeira *et al.*, 2012).

The present study was carried out to evaluate the essential oils of 3 Lauraceae species for their use in dermocosmetics. Moreover, the chemical composition of the essential oils was identified and the antioxidant and cytotoxic capacity, as well as the inhibition of tyrosinase and elastase activity were investigated.

MATERIAL AND METHODS

Plant material

Lauraceae species were collected at the Adolpho Ducke Forest Reserve in Manaus – Amazonas, Brazil. Exsiccates of *O. ceanothifolia* – INPA 189734, *O. leucoxylo* – INPA 177304 and *O. minor* – INPA 189746 were deposited in the herbarium at the National Institute of Amazonian Research (INPA) and were identified using the Ducke Reserve Flora Project (Ribeiro *et al.*, 1999).

Essential oil extraction

Samples were oven-dried and ground in a knife mill. The essential oils were then separately submitted to hydrodistillation (4 h) using a modified Clevenger-type apparatus. Volatile oils were distilled over water, collected and dried over anhydrous sodium sulfate (Merck), then stored at -5°C until analysis. Yields were calculated using the ratio of oil mass obtained to the plant material mass used in the extraction. Results were expressed as percentages and all experiments were performed in triplicates.

Chromatographic analysis

Chromatographic characterization was performed using a Shimadzu® gas chromatograph (GC 2010) with a flame ionization detector (GC-FID) and a CP-Sil 5 CB column (100% dimethylpolysiloxane) from Varian®, measuring 15 m x 0.25 mm with 0.25 µm film thickness. A QP-2010 Chromatograph from Shimadzu® was also used, along with a mass spectrometer detector (GC-MS) and a VF1MS column from Varian®, with the same dimensions used for the GC-FID analysis. Analyses were run under the following conditions: for the mobile phase, helium (He) was used as a carrier gas with flow rate

of 2.0 mL/min; the injection was done with split ratio of 1:10, with injection port temperature at 250°C and detector at 290°C. The oven program was raised from 60°C to 240°C at 3°C/min. Analysis using GC-MS was applied using an electron impact technique at 70 eV and run under the same conditions used for GC-FID.

Identification of components

Individual identification of constituents was achieved by comparing Retention Indices (RI) obtained from the GC-FID with data from the literature (Adams, 2007) and by comparing the fragmentation patterns of mass spectra with the ones from the Wiley Registry of Mass Spectral Data. RIs were calculated by comparing Retention Times with a homologous series of n-alkanes (C₉ - C₂₂) in the equation of Van den Dool & Kratz (1963). Concentrations of the components were calculated using the individual GC-FID peak area for each substance.

Free radical scavenging activity

Antioxidant potential was assessed by measuring the scavenging capacity of the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), following the methodology used by Molyneux (2004) with modifications. Initially, 270 µL of DPPH (1 mg/mL) and 30 µL of the sample solutions at a concentration of 100 µg/mL were added to each well of a 96-well microplate. Trolox[®] was used as a positive control and DMSO was used as a negative control. The plate was incubated for 30 min at room temperature in a dark environment and absorbance was read at 517 nm. Analyses were performed in triplicate and results were expressed in percentage of DPPH radical capture, according to the formula %AA = [100 - (Abs_{sample} / Abs_{control})*100], where Abs_{control} is initial absorbance of the ethanolic solution of DPPH and Abs_{sample} the absorbance of the reaction mix (DPPH + sample).

Tyrosinase inhibition test

Tyrosinase inhibition was tested according to the method described by Chan *et al.* (2009). A solution of the essential oil at 1 mg/mL was prepared using DMSO as a solvent. Triplicates of 20 µL of each oil solution and 80 µL of tyrosinase solution (0.1 mg/mL) were pipetted into a 96-well microplate. The initial absorbance (A1) was determined at 475 nm using a DTX 800 microplate reader, Beckman. The plate was incubated for 5 min at 37°C, and then 100 µL of DOPA (dihydroxyphenylalanine) at 0.5 mg/mL

was added. The plate was then incubated for 15 min at 37°C and the final absorbance (A2) was determined using the same wavelength. Kojic acid was prepared in the same way as the samples and used as a standard control. All analyses were performed in triplicate. CI₅₀ was calculated using the following formula in the programs Microsoft Excel 2010 and OriginPro8: % inhibition = [100 - (Abs_{T₂₀sample} - Abs_{T₀sample} / Abs_{T₂₀control} - Abs_{T₀control}) x 100], where Abs_{T₀} = initial absorbance (time: 0 min) and Abs_{T₂₀} = final absorbance (time: 20 min).

Elastase inhibition test

Elastase inhibition was assessed according to the method described by Kim *et al.* (2004). First, 60 mM Tris-HCl (pH 7.5) and a 5% DMSO buffer were prepared. The porcine pancreatic elastase (PE – E.C.3.4.21.36) was dissolved in distilled water to obtain a stock solution of 1:10. The substrate N-Succinyl-Ala-Ala-Ala-p-nitroanilide (AAAPVN) was dissolved in the buffer at 10 mM. Gallic acid and ellagic acid were used as a positive control and distilled water was used as a negative control. The essential oils, standard (positive control) and blank (negative control) were incubated with the enzyme and buffer in 96-well microplates for 5 min in an incubator at 37°C. After this period, 25 µL of AAAPVN was added and the samples had their absorbance read at 405 nm (A1). Next, the plate was once again placed in the incubator for 1 h at 37°C. After the incubation period, a second reading (A2) was performed at the same wavelength. The calculation was done according to the equation: % inhibition = 100 - (A2 - A1 sample) x 100 / A2 - A1 control.

Cytotoxicity assay

To test the cytotoxicity of the oils, an evaluation of the hemolytic potential was carried out according to the method described by Jimenez *et al.* (2003). Analyses were performed in triplicate using a 2% mouse erythrocytes suspension in a solution of 0.85% NaCl containing 10 mM CaCl₂ added to the wells of a 96-well microplate. Oils were then added at concentration of 1.22-625 µg/mL. After incubation at room temperature for 30 min and centrifugation at 450 g for 10 min, the supernatant was removed and the hemoglobin was measured using a spectrophotometer reader at 540 nm.

RESULTS AND DISCUSSION

Lauraceae are internationally known for having

aromatic species and *Ocotea* is one of the most complex genera of this family (Antonio *et al.*, 2020). After centuries of uncontrolled exploration in the Amazon region leading to the identification and exploitation of many species, essential oil-producing species continue to have economic interest, though few chemical and pharmacological studies have been published in the literature despite the number of species of interest.

The yield of oils obtained using hydrodistillation in this study varied from 0.02% to 0.13%. In all cases, leaves produced more oil than barks and stems. All three *Ocotea* species presented similar average yields with a mean percentage of 0.12 ± 0.1 for leaves and 0.05 ± 0.01 in stems. Yields were significantly different from what has been reported in the literature for other species of the same genus, such as *O. limae* (1.0%), *O. gardineri* (0.9%), *O. nigrescens* (0.23%), *O. splendens* (0.35%), *O. duckei* (0.70%), *O. puchury-major* (1.5%) and *O. caudata* (0.37%) (Barbosa-Filho *et al.*, 2008; Yamaguchi *et al.*, 2013; Gil *et al.* 2016; Moraes *et al.*, 2017).

The yields obtained in this investigation are from a single sampling expedition and the difference in yields between the species examined here and in other publications may be explained by the influence of abiotic factors, including temperature, luminosity, seasonality, nutrition and water. Future studies of *Ocotea* species are encouraged in order to optimize the yield, thus the variation that can occur during different seasons should be assessed, taking into consideration the prevalent seasons in the Amazon region, as well as the optimization of extraction by altering the proportions of water, temperature, and mass/volume (Barbosa *et al.*, 2012).

Twenty-five components of the essential oils were identified, the descriptions of which are given in Table No. 1. The presence of β -caryophyllene was detected in significant quantities in all seven oils analyzed, being one of the three main components found in stems and leaves of *O. leucoxyton* (8.90% and 10.67%) and *O. minor* (18.67% and 7.91%), respectively. The other major components were caryophyllene oxide in stems (16.01%) and leaves (17.53%) of *O. leucoxyton*, stems (15.74%) of *O. minor* and stems (16,9%) and leaves (22.37%) of *O. ceanothifolia*, and spathulenol in leaves (13.37%) and barks (6.13%) of *O. minor*.

The chemical profile of the essential oils revealed a predominance of sesquiterpenes, especially in the form of hydrocarbons. This profile is in agreement with what has been reported in the

literature for other species of this family (Gottlieb *et al.*, 1981; Takaku *et al.*, 2007).

According to reported data, the chemical composition of essential oils extracted from different plant parts of the *Ocotea* species display a profile rich in monoterpenes, sesquiterpenes and phenylpropanoids. Similarly, this study also detected a profile rich in sesquiterpenes and monoterpenes. The alpha-pinene, found in the oils of *O. ceanothifolia* and *O. leucoxyton*, was also one of the key components found in the *Ocotea* sp. oil reported by Olivero-Verbel *et al.* (2010), which presented antioxidant and cytotoxic activities. The chemical composition of oils from the *Ocotea* sp. in the present study are in accordance with what has been found in other *Ocotea* species, where there is the presence of the monoterpenes linalool and pinene, and the sesquiterpenes, caryophyllene and germacrene D (Menut *et al.*, 2002).

However, some different chemical characteristics from what has previously been reported for *Ocotea* were observed, such as the absence of benzenoids and phenylpropanoids like safrole, methyl eugenol and cinnamaldehyde, which were found in species like *O. odorifera*, *O. pretiosa* and *O. sassafras* (Chaverri *et al.*, 2011). In the screening done by Takaku *et al.* (2007) with essential oils from 10 species of *Ocoteae* from Costa Rica, such components were also not reported.

Linalool was one of the constituents found in all species, however its presence was only unanimous in stems, which was not the case in leaves. This particularity can also be found in other species of Lauraceae, where the compound was found solely or mostly in stems (Chaverri & Ciccio, 2008; Alcântara *et al.*, 2010). This monoterpene is of particular importance to the cosmetics industry, as it is commonly used as a perfume fixative. This interest has led to the uncontrolled extractivism of *Aniba rosaeodora*, which harbors a linalool content of 74 to 96%, and is now considered at risk of extinction. Linalool is a tertiary alcoholic monoterpene of open chain. It can be found regularly as a mixture of position isomers and has multiple biological activities that can be associated with its presence in the composition of the Lauraceae essential oils, such as insecticide, antimicrobial and anti-inflammatory activities (Kraiovic *et al.*, 2018).

Caryophyllene was a major component in the essential oils of the analyzed species. This volatile metabolite has a strong smell and is used in traditional medicine possessing many biological

Table No. 1
Percentage (%) of essential oil components in *Ocotea leucoxylo*, *O. minor* and *O. ceanothifolia*

Compound	RI	<i>Ocotea leucoxylo</i>		<i>Ocotea minor</i>			<i>Ocotea ceanothifolia</i>	
		Leaves	Stems	Leaves	Stems	Bark	Leaves	Stems
Alpha pinene	934	7.71	-	-	-	-	1.24	1.22
Beta pinene	978	7.92	-	-	-	-	-	-
Linalool	1099	-	4.76	-	3.10	-	-	3.42
Methyl geranate	1321	-	-	-	-	-	4.16	6.87
Alpha cubebene	1353	2.23	1.57	-	1.77	-	-	-
Alpha copaene	1380	10.11	2.10	-	-	-	-	-
Beta cubebene	1394	2.61	-	1.60	-	-	-	-
Beta elemene	1395	1.90	-	-	-	-	2.16	-
Beta caryophyllene	1424	8.90	10.67	18.69	7.91	4.01	3.95	1.46
Alpha humulene	1458	2.48	2.46	-	1.29	-	-	-
Alpha amorphene	1480	3.96	2.80	3.11	-	1.46	-	-
Germacrene D	1480	-	2.31	4.27	1.49	-	3.88	1.80
Beta selinene	1491	3.30	2.23	6.24	-	1.98	3.05	7.55
Valencene	1498	2.55	5.12	-	-	2.67	-	-
Bicyclogermacrene	1501	-	-	8.55	-	-	-	-
Alpha muurolene	1504	-	-	1.51	1.71	1.32	-	-
Gamma cadinene	1518	4.28	3.86	-	-	3.16	-	-
Delta cadinene	1527	1.50	7.72	-	4.19	7.49	-	-
Elemol	1552	-	1.76	-	-	2.15	-	-
Spathulenol	1582	5.78	10.45	13.37	6.63	6.13	22.23	7.66
Caryophyllene oxide	1588	16.01	17.53	-	15.74	-	22.37	16.9
Humulene oxide	1616	-	-	-	-	-	5.51	-
Caryophylla-4(12),8(13)-diene-5-beta-ol	1638	-	4.35	-	2.28	-	-	-
Beta eudesmol	1655	-	8.60	-	-	1.65	-	-
Hexadecanoic acid	1961	-	-	-	7.09	-	-	-
Total		81.24	88.29	57.34	53.2	32.02	68.55	46.88
Yield (%)		0.13	0.05	0.11	0.04	0.06	0.11	0.07

activities such as anti-inflammatory, antiallergenic, anesthetic, cardiovascular, antifungal and anticarcinogenic activities (Fernandes *et al.*, 2007; Barbosa-Filho *et al.*, 2008).

The essential oils did not present free radical scavenging activity in the biological evaluations. Stabilization percentages at a single concentration were less than 13%. Antioxidant activity can be evaluated using many protocols, DPPH stabilization being one of the most common and widely used *in vitro* tests due to its simplicity, speed and reproducibility. *Ocotea* species have been shown to have activity against oxidative stress and microbial infections, but most studies are from organic extracts

(Rodrigues *et al.*, 2019). However, though essential oils of Lauraceae species have presented the capacity to stabilize this radical, some authors suggest tests containing lipids or lipid emulsions, such as β -carotene, are more appropriate to evaluate less polar samples, since the substrate used is a lipid emulsion. Therefore, it is necessary to perform other methods in order to further elucidate this activity. Test results can be verified in Table N^o. 2.

Oils from stems of *O. ceanothifolia* at 50 μ g/mL presented an inhibition capacity of about 50% against the enzyme tyrosinase. Conversely, none of the essential oils were able to inhibit elastase activity more than 16% at a concentration of 50 μ g/mL.

When comparing these results with the standard, and with what has been described in the literature for other natural products, the essential oils in this study present only a moderate potential. At the

concentration tested, other essential oils and extracts have demonstrated an inhibition rate of up to 90% (Teixeira et al., 2012).

Table No. 2
Biological activities of essential oils from *Ocotea*

Species	Part	DPPH (100µg/mL)	Tirosinase (100µg/mL)	Elastase (50µg/mL)
<i>O. ceanothifolia</i>	Leaves	8.73	<1	9.3
<i>O. ceanothifolia</i>	Stems	11.13	49.08	11.1
<i>O. leucoxydon</i>	Leaves	13.04	<1	16.3
<i>O. leucoxydon</i>	Stems	11.77	<1	7.7
<i>O. minor</i>	Bark	10.01	<1	8.3
Standard		100	90%	100%

With regards to cytotoxicity, an *in vitro* test observing the hemolytic activity on erythrocytes of mice was performed using the essential oils at concentrations between 1.22 - 625 µg/mL. Hemolysis was not observed for any of the oils, suggesting that the oils have low toxicity and the compounds present do not possess hemolytic activity or damage cell membranes. Evaluation of cell rupture is important when assessing raw materials for use in cosmetics, in order to define the potential of degradation or cell death provoked by the constituent material. Positive cytotoxicity results mean the cosmetic could possibly cause irritation to users; therefore, this test is often applied as a safety parameter for the use of these compounds.

Essential oils of Lauraceae are currently being used in cosmetics at both popular and industrial levels. The bark of *Aniba canelilla* is used in the perfume industry and its main constituents are reported to be the nitro-derived compounds 1-nitro-2-phenylethane and methyl eugenol. Stems and leaves of *A. rosaeodora* are used in shampoos, conditioners and perfumes and as a perfume fixative, with the precursor of linalyl acetate (linalool) as one of its main components. *Licaria puchury-major* presents a composition rich in safrole and eucalyptol and is used in the perfume and fragrance industry as a fixative or as raw material for its fabrication, it is also commonly used in dental products such as toothpaste

and mouth wash. *Ocotea cymbarum* is also rich in safrole and is often used in the production of perfumes and fragrances.

This study contributes to the chemical profile identification of essential oils from *O. minor*, *O. ceanothifolia* and *O. leucoxydon*. In addition, evaluation of the antioxidant, enzymatic and cytotoxic activities were achieved providing more information between potential activities based on the composition, which can help direct future chemical investigations, as well as contributing to the genus triage of promising species. Considering the chemical composition that was described and the biological activities reported here for the first time, the oils analyzed in this study are encouraging for future research regarding its applications in cosmetics and other biological activities.

CONCLUSION

The present study established the composition of essential oils from three species of Lauraceae, *O. minor*, *O. ceanothifolia* and *O. leucoxydon*, rich in terpenes of the sesquiterpene kind and to a lesser extent, of monoterpenes. This is the first account of the chemical composition and biologic activities of these seven essential oils, stimulating the development of new studies involving advanced biological activities and the future commercial use of these species.

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