



Chemical and Biological Evaluation of Essential Oils with Economic Value from Lauraceae Species

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Este trabalho compara pela primeira vez a composição química do óleo essencial das folhas de *Licaria canella* coletadas em duas estações climáticas. Os resultados foram comparados com os obtidos para o óleo essencial das folhas de outra espécie da família Lauraceae, *Aniba canelilla*, coletada no mesmo período. Ambos os óleos essenciais foram analisados por CG-DIC e CG-EM, e os resultados indicaram uma grande quantidade de benzenóides, sendo o principal constituinte em *L. canella* o benzoato de benzila e para *A. canelilla*, o 1-nitro-2-feniletano. A comparação das atividades biológicas mostrou que o óleo de *L. canella* (IC_{50} 19 $\mu\text{g mL}^{-1}$) foi mais ativo contra as cepas de *Leishmania amazonensis* e menos citotóxico em cultura de macrófagos do que o de *A. canelilla* (IC_{50} 40 $\mu\text{g mL}^{-1}$). Por outro lado, o óleo de *L. canella* exibiu uma maior citotoxicidade contra *Artemia salina* com uma concentração letal (CL_{50}) igual a 5,25 $\mu\text{g mL}^{-1}$.

This work compares the chemical composition of the essential oils from the leaves of *Licaria canella* collected in two different seasons. The results of this investigation were compared with the leaf essential oils of other species of the Lauraceae family, *Aniba canelilla*, collected at the same time. Both essential oils were analyzed by GC-FID and GC-MS. The results demonstrated a larger predominance of benzenoids, being the main constituent benzyl benzoate for *L. canella* and 1-nitro-2-phenylethane for *A. canelilla*. The comparison of the biological activities showed that *L. canella* (IC_{50} 19 $\mu\text{g mL}^{-1}$) was more active against *Leishmania amazonensis* strains and less cytotoxic in macrophage cultures than *A. canelilla* (IC_{50} 40 $\mu\text{g mL}^{-1}$). On the other hand, the *L. canella* oil displayed a higher cytotoxicity against *Artemia salina* with a lethal concentration (LC_{50}), equal to 5.25 $\mu\text{g mL}^{-1}$.

Keywords: *Licaria canella*, *Aniba canelilla*, essential oil, *Leishmania amazonensis*, cytotoxicity

Introduction

The Lauraceae family contains about 50 genera and approximately 2500-3500 species distributed in tropical to subtropical areas, with a few occurrences in temperate regions and is among those with the greatest number of specimens in different regions of the Amazon.¹ Twenty-two genera are found in Brazil, distributed in rain forests as well as in restingas and cerrados.² The floristic mapping held in the Adolpho Ducke Reserve (Amazonas

State, Brazil) cataloged so far 13 genera and 99 species.³ Most of the family members are characterized by a woody habit and are of great economic importance worldwide, as they provide valuable timber, aromatic oils and important substances that are widely employed in the pharmaceutical and food industries, with emphasis on the genera *Aniba*, *Licaria*, *Nectandra*, *Ocotea*. In the Amazonian region these genera are popularly known as “pau-rosa” (rosewood) and “louros”. The oil of “pau-rosa” (*Aniba rosaeodora* Ducke) has historical importance. It has already emerged in the third position in the exports lines of the Amazonian region, behind

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only rubber and chestnut, that rank first and second, respectively.⁴ *Licaria canella* (Meissner) Kostermans is a botanical species popularly known as “louro-pirarucu”. Within the ethnic group Tacana of the Amazonian region, this species has the same name and use as *Aniba canelilla* (H.B.K.) Mez, probably due to their aromatic barks.⁵ The barks of both species have ethnopharmacological use to alleviate abdominal pain, intestinal cramps or discomfort, without diarrhea.⁶ Phytochemical studies of the trunk wood of *L. canella* described the presence of dillapiol, elemicin and the neolignans canellins A-C.⁷ The ethanol extract of the bark of this species showed activity *in vitro* against chloroquine sensitive *Plasmodium falciparum* ($IC_{50} = 3.8 \mu\text{g mL}^{-1}$) and also resistant strains ($IC_{50} = 3.2 \mu\text{g mL}^{-1}$).⁶ The extract of the stem demonstrated low activity against RPMI 8226 cancer cells.⁸

Aniba canelilla, known as “casca-preciosa” (precious bark), was the target of an ancestral confusion in the search for the “country of cinnamon”.⁹ The cinnamon flavor was responsible for the confusion between *A. canelilla* and the *Cinnamomum* species. In the case of the bark of *C. zeylanicum* Blume, the major principle of cinnamon odor is cinnamic aldehyde,¹⁰ while in *A. canelilla*, it is the 1-nitro-2-phenylethane.¹¹ Previous works on *A. canelilla* described the isolation of benzyltetrahydroisoquinoline alkaloids from the stem bark.¹² The chemical compositions of the essential oils and hexane extract from the leaves and stem bark were identified by spectroscopic methods.^{13,14} Crude extracts of the stem bark from *A. canelilla* were active at $100 \mu\text{g mL}^{-1}$ *in vitro* against *Leishmania* spp. and *Trypanosoma cruzi*.¹⁵ The bark oil of this species showed cardiovascular effects in normotensive rats.¹⁶ A higher antioxidant activity using DPPH radical scavenging ($EC_{50} = 4.41 \mu\text{g mL}^{-1}$) was reported for methanol extracts of the wood. The brine shrimp bioassay performed with the trunk oils of *A. canelilla* ($LC_{50} = 21.61 \mu\text{g mL}^{-1}$) showed cytotoxicity higher than the wood extracts ($LC_{50} = 91.38 \mu\text{g mL}^{-1}$).¹⁷ 1-Nitro-2-phenylethane produced antinociceptive effects but its mechanism of action was not elucidated.¹⁸

This report presents the chemical composition of the essential oils of *L. canella* and relates it to the oil of *A. canelilla*, collected in two different seasons. The emergence of parasite resistance to current therapies highlights the importance of essential oils as novel antiparasitic agents. In this context, the oils collected in October 2007 were tested *in vitro* on promastigote forms of *Leishmania amazonensis*. Additionally, toxicity evaluation of these oils with non-parasite macrophages and brine shrimp (*Artemia salina*) were performed.

Experimental

Plant Material

The leaves of *L. canella* and *A. canelilla* were collected from four individuals at the Adolpho Ducke Reserve, near the city of Manaus, Amazonas state, Brazil. Voucher specimens were deposited in the Herbarium of INPA (Amazonas state) under numbers 226360 and 220094, respectively. These species were collected in the dry (October 2007) and rainy seasons (February 2008). Flowers were observed in February, without fruits in either month. Data of pluviometric precipitation were obtained at the site of the National Institute of Meteorology and these maps showed no atypical differences in these seasons.¹⁹

Extraction of essential oil

The leaves collected from *L. canella* and *A. canelilla* (600 g) were dried at room temperature for 3 days, minced and submitted to hydrodistillation for 4 h in a Clevenger-type apparatus.

Essential oil analysis

The identification of compounds was performed by comparison of their retention indices and mass spectra with those reported in the literature or stored in the Wiley data system library.^{20,21} The retention indices were calculated for all volatile constituents using *n*-alkane homologous series. GC analyses were performed using a HP 5890 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm) and a FID detector. The oven temperature was programmed from 60 °C to 290 °C at a rate of 3 °C min^{-1} , then isothermal at 290 °C for 10 min, using H_2 as the carrier gas (1.0 mL min^{-1}). Injector and detector temperatures were 230 °C and 280 °C, respectively.

GC-MS analyses were performed using a HP 6890 gas chromatograph interfaced with a HP 5873 Mass Selective Detector (ionization voltage 70 eV), equipped with a DB-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm), using He as the carrier gas (1.0 mL min^{-1}). Oven and injector temperatures were as described before.

In vitro antileishmanial assay

Promastigotes of *Leishmania amazonensis* strains MHOM/BR/77LTB0016, isolated from patients with cutaneous leishmaniasis in Manaus, were routinely cultured at 26 °C in Schneider’s medium supplemented

with 10% fetal calf serum (FCS), pH 7.2. Parasites were harvested from the medium on day 4, when a high percentage of infective forms (metacyclic promastigotes) were found. After being harvested, the parasites were counted in a Neubauer's chamber and adjusted to a concentration of 4×10^6 promastigotes mL^{-1} using the supernatant of each culture as diluent. The samples were dissolved in DMSO (the highest concentration was 1.4%, which was not hazardous to the parasites) and added to parasite suspensions in final concentrations between 0.156 to 320 $\mu\text{g mL}^{-1}$. After 24 h of incubation, the parasites were counted and compared to the controls, containing only DMSO and parasites. Pentamidine isethionate was used as the reference drug. The sample concentration corresponding to 50% of parasite growth inhibition was expressed as the IC_{50} .

Cytotoxicity assay

In order to evaluate the toxicity of the sample for the host cell, mice peritoneal macrophages were isolated in RPMI 1640 medium (Sigma Cell Culture, St. Louis, MO, USA), containing 200 UI mL^{-1} penicillin, 200 $\mu\text{g mL}^{-1}$ of streptomycin, 1 mmol L^{-1} sodium pyruvate, 1 mmol L^{-1} of L-glutamine and 1 mol L^{-1} HEPES buffer (Sigma Cell Culture, St. Louis, MO, USA). Cells were counted in a Neubauer's chamber using Erythrosine B as vital dye (Sigma Cell Culture, St. Louis, MO, U.S.A.) and adjusted to a concentration of 4×10^6 mL^{-1} . After that, the cells were cultured in a 96 well culture plate (Falcon, New Jersey, U.S.A.), at 37 °C and in an atmosphere of 5% CO_2 . The sample was added to the medium in a concentration equivalent to IC_{50} and $2 \times \text{IC}_{50}$ of the *in vitro* activity assay from *L. amazonensis* species. The sample and pentamidine isethionate (reference drug) were added to the cultures and after 24 hours the viability of treated cells were compared to the control without drugs, through the MTT methodology.^{22,23}

Toxicity testing against brine shrimp

The brine shrimp (*Artemia salina*) lethality test was performed by the method of Meyer *et al.*²⁴ with some modifications. The samples were dissolved in dimethylsulfoxide (DMSO) and diluted serially in seawater. In each case three replicates of each concentration were assayed. Survivors were counted after 24 h and the LC_{50} values in $\mu\text{g mL}^{-1}$ were determined by Probit analysis.²⁵ Saline solution with DMSO was used as negative control ($\text{LC}_{50} > 1000 \mu\text{g mL}^{-1}$), while lapachol was used as a positive control ($\text{LC}_{50} = 23.0 \mu\text{g mL}^{-1}$).

Results and Discussion

The yield of the oils was 1.2% (October, dry season) and 1.3% (February, rainy season) for *L. canella* leaves. The extraction resulting from *A. canelilla* leaves was 0.8% (October) and 0.9% (February). As shown in Table 1, compounds of the essential oils of *L. canella* and *A. canelilla* were identified by GC-FID and GC-MS analyses.

The results showed that the essential oils of these species were rich in benzenoid compounds, with minor constituents represented by mono and sesquiterpenoids (Table 2).

The major constituent of the leaf essential oils from *L. canella* (October 2007, 69.7% and February 2008, 73.0%) was benzyl benzoate. For *A. canelilla* it was 1-nitro-2-phenylethane (October 2007, 88.9% and February 2008, 88.5%). The percent content of this last compound, which was recently isolated and identified by NMR spectral data,¹⁷ is in agreement with previous studies published on this species collected in the rainy season.²⁶ On the other hand, in contrast with the results described for the species collected in the State of Pará,²⁶ the collection related to the dry season (October 2007) did not show a significant difference in its content of 1-nitro-2-phenylethane when compared to the rainy season. Additionally, the presence of methyleugenol, a component considered as a marker of the oils from Pará State,²⁶ was not identified in the analyses of the essential oils of *A. canelilla* found in Amazon State. The qualitative comparison and the percent content of the oils from the two collections showed similarity, (*E*)-caryophyllene being the second most abundant constituent in both collections. With respect to the essential oils from the leaves of *L. canella*, there is no report on the description of its chemical composition, which presented almost the same profile in both extractions, with benzyl benzoate being the main constituent. The other more abundant constituents were: α -pinene, α -phellandrene, α -copaene and (*E*)-caryophyllene. *p*-cymene, δ -3-carene and 1,8-cineol were detected only in the essential oils of the rainy season. The percent differences are listed in Table 1.

The evaluation of the antileishmanial activity of the essential oils (October 2007) of *L. canella* ($\text{IC}_{50} = 19 \mu\text{g mL}^{-1}$), and *A. canelilla* ($\text{IC}_{50} = 40 \mu\text{g mL}^{-1}$) indicate moderate activity against *L. amazonensis* promastigotes. These essential oils presented low cytotoxicity on uninfected peritoneal macrophages, even when evaluated in amounts twice as large as their IC_{50} and comparable to the reference drug (pentamidine) (Table 3). These results are relevant due to the low toxicity presented by the oils when compared to the high toxicity of the therapeutic drugs used to treat the disease.²⁷

Table 1. Chemical composition of the leaf essential oils of *L. canella* and *A. canelilla*

Compounds	RI	Oil composition / (%)			
		<i>L. canella</i>		<i>A. canelilla</i>	
		October	February	October	February
α -thujene	930	0.17	0.15	-	-
α -pinene	939	3.54	3.05	0.75	0.61
camphene	954	1.54	1.41	-	-
β -pinene	982	1.35	1.31	0.43	0.46
β -myrcene	991	0.35	0.45	-	-
α -phellandrene	1003	4.20	3.33	-	-
δ -3-carene	1010	-	1.60	-	-
p-cymene	1025	-	0.26	-	-
β -phellandrene	1030	-	-	0.80	1.14
benzyl alcohol	1032	0.34	Tr	-	-
1,8-cineol	1033	-	0.19	-	-
(E)- β -ocimene	1048	tr	Tr	-	-
γ -terpinene	1061	tr	Tr	-	-
α -terpinolene	1088	0.39	0.30	-	-
linalool	1097	-	-	0.23	0.23
borneol	1164	0.48	0.47	-	-
terpin-4-ol	1175	tr	Tr	-	-
α -terpineol	1189	-	-	0.23	0.29
<i>trans</i> -cinnamaldehyde	1266	tr	0.78	-	-
bicycloelemene	1282	0.83	0.19	-	-
1-nitro-2-phenylethane	1327	-	-	88.9	88.5
eugenol	1356	0.42	0.28	0.17	0.08
α -cubebene	1378	0.27	0.11	-	-
α -copaene	1380	4.99	4.51	0.71	0.71
β -cubebene	1390	0.60	0.46	-	-
(Z)-caryophyllene	1405	-	-	0.30	0.27
(E)-caryophyllene	1424	3.01	3.02	4.21	5.04
<i>trans</i> -cinnamylacetate	1443	0.81	0.83	-	-
α -humulene	1454	0.79	0.71	0.46	0.64
epi-bicyclosesquiphellandrene	1489	0.12	-	-	-
bicyclogermacrene	1494	0.85	1.54	-	-
β -bisabolene	1508	-	-	0.49	0.64
δ -cadinene	1528	0.37	0.22	0.14	0.20
cadina-1,4-diene	1529	0.10	-	-	-
spathulenol	1578	0.11	-	-	-
caryophyllene oxide	1581	-	-	0.14	0.91
germacrene D	1584	0.26	0.28	-	-
benzyl benzoate	1762	69.7	73.0	0.21	0.19
Identified components / (%)		95.6	98.7	98.1	99.9

tr: traces (< 0.1%).

Table 2. The chemical class distribution of the essential oils of *L. canella* and *A. canelilla*

Compound class	<i>L. canella</i> / (%)		<i>A. canelilla</i> / (%)	
	October	February	October	February
Monoterpenoids	12.0	12.5	2.4	2.7
Sesquiterpenoids	12.3	11.2	6.4	8.4
Benzenoids	71.3	74.9	89.3	88.8

Table 3. *In vitro* activity of essential oils of *L. canella* and *A. canelilla* against *L. amazonensis* and macrophages

Sample	IC ₅₀ / (µg mL ⁻¹)	Cytotoxicity / (%)	
	<i>L. amazonensis</i>	Mice (Balb/c)	macrophages
<i>L. canella</i>	19.0 ± 0.9	IC ₅₀	6.2
		(2 × IC ₅₀)	15.1
<i>A. canelilla</i>	40.0 ± 1.2	IC ₅₀	9.3
		(2 × IC ₅₀)	16.0
Pentamidine (reference drug)	4.8 ± 0.1		24.4

In this context, the results obtained in the evaluation with *Artemia salina* indicated high cytotoxicity for *L. canella*, with a lethal concentration (LC₅₀) equal to 5.25 µg mL⁻¹, while in the case of *A. canelilla*, the LC₅₀ observed was about 13 times greater (68.37 µg mL⁻¹), being thus in agreement with the greater activity against *L. amazonensis* strains found for the oils of *L. canella*.

Conclusions

The main constituent of the essential oils of *L. canella* is commercially used as a topical medication against several parasitoses, which suggests a potential use of this oil for this purpose. The leaves of *L. canella* and *A. canelilla* may be qualified as two natural, abundant and ecologically renewable sources of benzyl benzoate and 1-nitro-2-phenylethane, respectively, with commercial value for medicinal purposes in the case of *L. canella*^{28,29} and as an aroma for the cosmetic and food industries regarding *A. canelilla*.

Acknowledgments

The authors are grateful for the financial support provided by CNPq and by the FAPEAM / PIPT Program and thank Ms. June Justa for revising the manuscript.

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Received: January 31, 2009

Web Release Date: June 18, 2009