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Essential oils of *Protium spp*. samples from Amazonian popular markets: chemical composition, physicochemical parameters and antimicrobial activity

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Species belonging to the Burseraceae family, especially of the genus *Protium*, are well known for producing fragrant oleoresins known in Brazil as *breus*. In this study, the physicochemical properties and antimicrobial activities of essential oils obtained from commercials samples of *breu* from the Brazilian Amazon were evaluated. Essential oils were extracted by hydrodistillation using a modified Clevenger apparatus and analyzed by gas chromatography/mass spectrometry (GC/MS) and GC/flame ionization detector (GC/FID). *p*-Cymene was the major constituent in all of the analyzed *breu* samples, with concentrations ranging from 21.9% to 51.9%, except for the white *breu* sample from Adolfo Lisboa Popular Market (ALPM), which contained α-pinene (22.7%). Other common monoterpenes were β-phellandrene, α-phellandrene, β-pinene, *trans*-dihydro-α-terpineol, α-terpineol and α-terpinene. The refractive indices ranged from 1.4769 to 1.4849 and the optical rotation ranged from −14.15° to +29.96°. The antimicrobial activity was low for all of the samples. The essential oil of black *breu* from ALPM was the only sample exhibiting antimycobacterial activity.

Keywords: *Protium*; Burseraceae; *breu*; *p*-cymene; essential oil composition

Introduction

The Burseraceae family includes eighteen genera with about 700 species divided into three tribes, Canarieae, Protieae and Bursereae. This family is spread over low latitude regions without intense cold, including rainforests, dry forests and deserts (1). The trees in this family range in size from small large, with some species reaching the forest canopy. Species belonging to this family are well known for producing fragrant exudates with economic, medicinal and cultural value (2). The abundant fluid secretions come from glands and channels located primarily in the bark but also deeper in the trunk. These secretory channels can remain with the plant for life or can arise from external causes, such as injuries due to trauma, burns or incisions that can lead to pathological or physiological resin leakages (3).

Species belonging to the genus *Protium* produce a resinous exudate, known as elemi, after an injury in the trunk of the plant caused by a fly. In Brazil, this type of elemi is known as *breu*. A volatile oil can be obtained from this exudate. Once out of the plant, the resin composition changes due to oxidation and the loss of volatiles, leading to changes in its physical characteristics from a soft and plastic appearance (soft elemi) to a harder, brittle and viscous material (hard elemi) (3).

Depending on the species of *Protium* from which it is extracted and its organoleptic characteristics, Amazonian elemi is commonly known as black or white *breu*. Its chemical composition is complex, comprising monoterpenes, sesquiterpenes and triterpenes in varying concentrations (4–7). Within this range of constituents, monoterpenes and sesquiterpenes with antimicrobial, antioxidant, analgesic, anti-inflammatory and antitumor activities have been found (5), (8–10).

Both white and black *breu* in the Amazon region of Brazil are used by the Quilombola communities of Oriximiná, State of Pará, for medicinal purposes. The *breu* is used to treat headaches by inhalation or in plasters against pain and inflammation (11). Moreover, it has been used in folk medicine against lung diseases, such as pneumonia and tuberculosis, as well as gonorrhea (3, 12). These data indicate potential antimicrobial activities of the resins.

However, the literature cited above used only discrete pure samples of *Protium*. To the best of our knowledge, no information on the chemical and biolog-

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ical properties of commercial *breu* samples, obtained from local markets, is available. Because the mixture of exudates from different areas and several *Protium* species varies and resins from others plants are also commonly used, variable compositions and properties are expected.

The aim of this study was to collect chemical, physicochemical and biological activity data from the essential oils of different commercial samples of *breu* to establish quality standards for these materials.

Experimental

Plant material

Ten *breu* samples were obtained from five Amazonian popular markets: six from the City of Manaus, Amazonas State [Adolpho Lisboa Popular Market (ALPM), Alvorada Fair (AF) and Parque 10 Fair (P10F)] and four from Pará State [Oriximiná Popular Market (OPM) and Ver-o-Peso Popular Market (VPM)].

Essential oil extraction

Commercial samples of white and black *breus* were extracted by hydrodistillation using a Clevenger-type apparatus for four hours. The extraction system was then turned off. The essential oil was separated from the hydrolate by decantation and centrifugation, and then stored under refrigeration in sealed amber flasks.

Gas chromatography analyses

Gas chromatographic (GC) analyses of the essential oils were carried out on an Agilent 7890A gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector (FID) and an HP-5 (5% phenyl, 95% methylsiloxane) fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The oven temperature was increased from 60° to 240°C at 3°C/minute. The injector temperature was kept at 250°C and the detector temperature at 280°C. A 1.0-μL sample of the oil, containing 1% dichloromethane, was injected with a split ratio of 1:20. Hydrogen was used as the carrier gas at 1.5 mL/minute.

Mass spectra were obtained with an Agilent 5973N system operating in electronic ionization mode (EI) at 70 eV, with a scan mass range of 40–450 *m/z* and a sampling rate of 3.15 scan/second. The ion source temperature was kept at 230°C, the mass analyzer at 150° C and the transfer line at 260°C. The mass detector was coupled to an Agilent 6890 gas chromatograph fitted with a low bleeding 5% phenyl, 95% methylsilicone (HP-5 mass spectrometer, 30 m × 0.25 mm i.d., 0.25 μm film thickness) fused silica capillary column. The injection procedure and oven temperature program were the same as described above. Helium was the carrier gas and flowed at 1.0 mL/minute.

Linear retention indices (LRI) were measured (13) by injection of a series of *n*-alkanes (C_7–C_{26}) using the same column and conditions as above for the GC analyses.

Component identification

Identification of the oil components was performed with a computer search using the Wiley library of mass spectral data (14) and by comparison of their calculated LRIs with literature data (15). The relative compositions were obtained from the FID.

Physicochemical analyses

The optical rotation was determined using a Perkin Elmer 341 digital polarimeter. The assay was performed in a thermostated 10 mm cell at 20°C and with monocromatic light at 589 nm. After each measurement, the cell was washed with acetone and dried. Refractive index was measured using a Carl Zeiss 120540 refractometer at 20°C. For each assay, 15 μL of the essential oil was used.

Determination of antimicrobial activity

The antimicrobial activity was determined by measuring the minimal inhibitory concentration (MIC) according to the international standard methodology of the Clinical and Laboratory Standards Institute (CLSI) (16). The microorganisms tested were the yeast-like fungus *Candida albicans* ATCC 10231 (fungal collection from Faculty of Odontology, Universidade Federal do Rio de Janeiro), the filamentous fungus *Aspergillus niger* ATCC 16404 (fungal collection from Clementino Fraga Filho Universitary Hospital, Universidade Federal do Rio de Janeiro), the Gram-positive bacterium *Staphylococcus aureus* MRSA (BMB9393) (Clementino Fraga Filho Universitary Hospital, Universidade Federal do Rio de Janeiro) and the Gram-negative bacterium *Escherichia coli* ATCC 8739. Positive controls were tested with 10 μL of reference antibiotics (1 mg/mL, Tween 80 (0,5% v/v) in water): amphotericin B and ciprofloxacin were tested against fungi and bacteria, respectively. Bacteria were previously grown on a brain heart infusion (BHI) agar solid medium at 37°C for 24 hours. Bacterial inocula were prepared with cell suspensions of 5×10^6 CFU/mL determined spectrophotometrically at 600 nm in Mueller–Hinton medium. To evaluate the antibacterial activity, 10 μL of the bacterial suspension was added to each well containing 110 μL of a particular dilution of the tested essential oil (5 to 0.05 mg/mL in serial dilution), reaching a final concentration of 5×10^5 CFU/mL. The yeast-like fungus was previously grown on Sabouraud agar medium at 37°C for 24 hours and the filamentous fungus was grown on potato agar (PDA) at room temperature for three days. Similarly to bacteria, the concentrations of *A. niger* conidia and *C. albicans* yeast in RPMI-MOPS

(pH 7.2) medium suspensions were determined spectrophotometrically at 530 nm. A 100-μL sample was added to each well containing 200 μL of a particular dilution of the tested essential oil (5 to 0.05 mg/mL in serial dilution) obtaining final concentrations in the range of 5×10^2 to 2.5×10^3 CFU/mL for *C. albicans* and 0.4×10^4 to 5×10^4 CFU/mL for *A. niger*. The MIC was determined visually by turbidity and by adding the redox indicator resazurine (0,005% in PBS pH 7.2).

In vitro antimycobacterial activity evaluation

All of the essential oils were screened against *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294 by a microdilution method using Alamar Blue as an indicator of cell viability (17). Six fixed concentrations of each essential oil were tested (100.0, 50.0, 25.0, 12.5, 6.25 and 3.12 μg/mL). Media plus bacteria, with or without rifampicin, were used as positive and negative controls, respectively. The MIC was determined for the active sample and the positive control.

Results and discussion

Yield of essential oils

Among the studied samples, the black *breu* from VPM, which showed no characteristic fragrance, and the white *breu* from ALPM, with a solid crystalline appearance, yellow color and distinctive fragrance, showed the most prominent organoleptic differences. The black *breu* from ALPM exhibited similar color and odor characteristics to some of the white *breu* samples, while the white *breu* from VPM was odorless and darker than the other white *breus*. Hydrodistillation of *breu* samples yielded fragrant, light yellow to yellow essential oils. The oil yields and their physicochemical properties are listed in Table 1. The highest white *breu* essential oil yields were from VPM (3.7%), while the lowest were from ALPM samples (0.3%). For the black *breus*, the highest yield was obtained from OPM samples (2.8%), while the lowest one was from VPM samples, with no oil at all. These wide yield variations are common and may be related to tampering or poor storage of the marketed product (18, 19).

Composition of essential oils

The chromatographic profile of each *breu* essential oil is presented in Figure 1, from which the most relevant constituents were identified. The compositions of the essential oils are presented in Table 2. The monoterpene *p*-cymene appears as the major constituent in all of the analyzed *breu* samples, with concentrations ranging from 21.9% to 51.9%. These data are in agreement with analyses of essential oils from some *Protium* species, including *P. heptaphyllum* subsp. *heptaphyllum* (18), *P. strumosum* (18–20), *P. icicariba* (7, 18), *P. hebetatum, P. paniculatum, P. paniculatum* var. *riedelianum*, *P. spruceanum, P. altsonii* and *P. nitidifolium* (19). The only exception was the white *breu* sample from ALPM, which contained α-pinene (22.7%) as the major constituent. Despite its organoleptic properties, suggesting adulteration involving *Protium* and Jatobá (*Hymenaea courbaril* – Caesalpiniaceae) resins, its chemical constitution is not in agreement with literature data (21). On the other hand, Case et al. (22) reported that the essential oil of *copal oro*, most likely obtained from *H. courbaril*, contained α-pinene (21.35%) as one of its major constituents. In a study about essential oils from different species of *Protium*, Silva (18) observed that essential oils derived from resins of *P. nitidifolium* and *P. divarictum* contained α-pinene as major constituent. According to Ramos et al. (19), some samples of species of *Protium*, such as *P. hebetatum and P. altisonii* may contain α-pinene as a major constituent depending on the time of collection. This monoterpene was present in the composition of all of the other samples (with a concentration ranging from 6.7% to 22.7%), with the exception of the white *breu* sample from P10F and the black *breu* sample from OPM. Another common monoterpene among the samples was β-phellandrene, found in the white and black *breus* from P10F and OPM and in the white *breu* from VPM. Alpha-phellandrene was present in the white *breu* samples from P10F and VPM and in the black *breu* samples from OPM. Other monoterpenes found were β-pinene and *trans*-dihydro-α-terpineol in the white *breu* from ALPM, α-terpineol in the white *breu* from OPM and α-terpinene in the black *breu* from OPM. Among the major constituents, the only sesquiterpene found

Table 1. Yield and physical properties of nine *breu* essential oils.

	White *breu*					Black *breu*			
Parameter	ALPM	AF	P10F	OPM	VPM	ALPM	AF	P10F	OPM
Yield (% v/w)	0.3	2.7	0.8	1.3	3.7	1.3	0.5	1.8	2.8
Refractive index*	1.4769	1.4820	1.4859	1.4811	1.4771	1.4831	1.4788	1.4781	1.4802
Optical rotation*	−14.15°	−1.91°	19.76°	5.97°	23.95	−5.27°	6.41°	25.43°	29.96°

Notes: *All measured at 20°C. ALPM, Adolpho Lisboa Popular Market; AF, Alvorada Fair; P10F, Parque 10 Fair; OPM, Oriximiná Popular Market; VPM, Ver-o-Peso Popular Market.

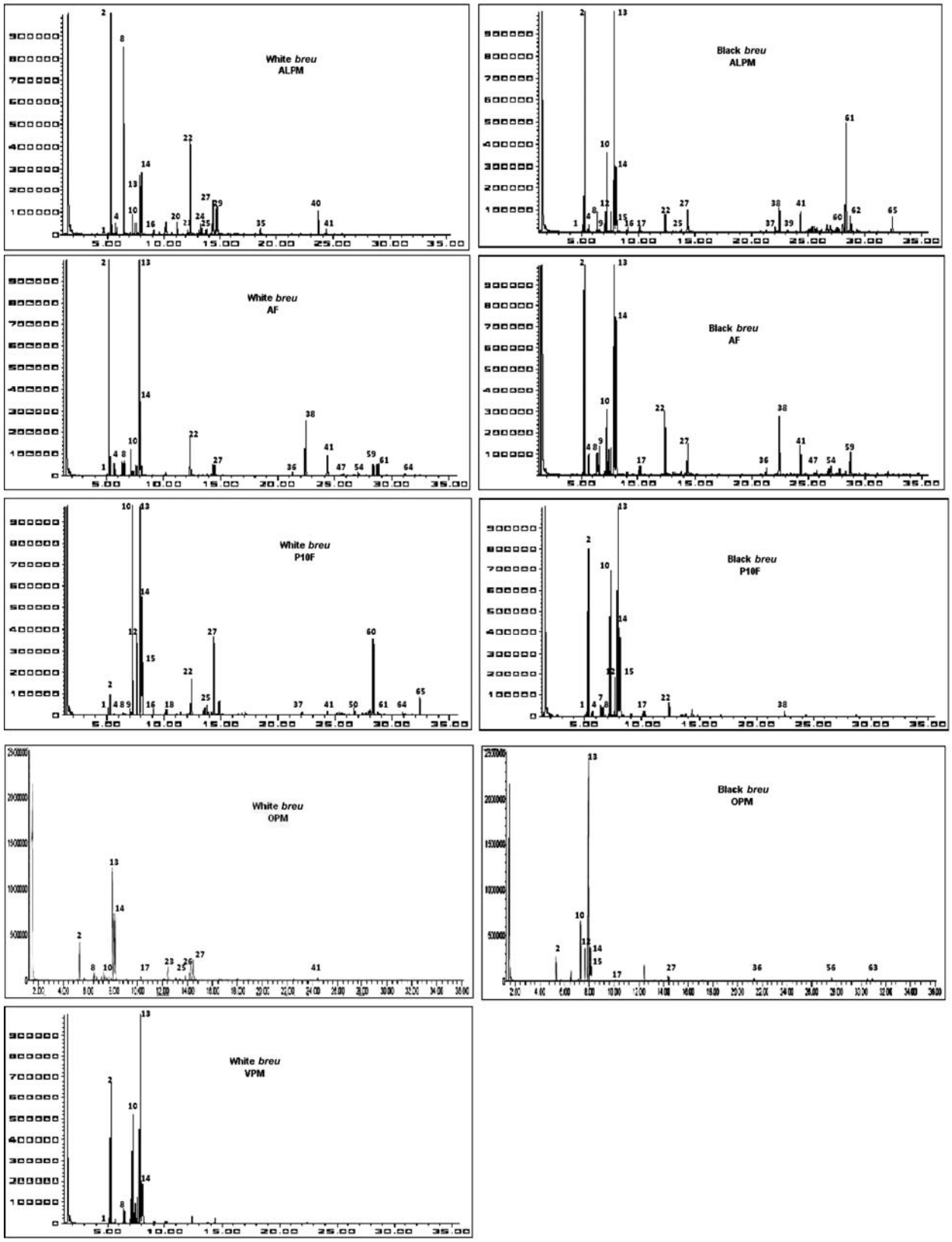

Figure 1. Gas chromatograms of the essential oil of different samples of *breu*. The different compounds are shown by Arabic numerals; **1**: α-thujene; **2**: α-pinene; **4**: camphene; **7**: *trans*-*p*-menthane; **8**: β-pinene; **9**: *p*-3-menthene; **10**: α-phellandrene; **12**: α-terpinene; **13**: *p*-cymene; **14**: β-phellandrene; **15**: 1,8-cineole; **16**: γ-terpinene; **17**: terpinolene; **18**: *p*-cymenene; **20**: endo-fenchol; **21**: trans-pinocarveol; **22**: trans-dihydro-α-terpineol; **23**: camphor; **24**: borneol; **25**: terpine-4-ol; **26**: cymen-8-ol; **27**: α-terpineol; **29**: methyl chavicol; **35**: isobornyl actetate; **36**: α-cubebene; **37**: cyclosativene; **38**: α-copaene; **39**: β-elemene; **40**: longifolene; **41**: β-caryophyllene; **47**: α-humulene; **50**: β-acoradiene; **54**: γ-himachalene; **56**: bicyclogermacrene; **59**: δ-amorphene; **60**: γ-cadinene; **61**: δ-cadinene; **62**: α-cadinene; **63**: spathulenol; **64**: caryophyllene oxide; **65**: 1,10-di-epi-cubenol.

Table 2. Chemical composition of *breu* essential oils obtained from commercial samples from the Amazon.

				Percentage								
				White *breu*					Black *breu*			
S.N	Compounds	RI^{lit}	RI*	ALPM	AF	P10F	OPM	VPM	ALPM	AF	P10F	OPM
1	α-Thujene	930	930	0.2	0.1	0.4	0.4	0.4	0.6	–	0.4	–
2	α-Pinene	939	938	22.7	17.7	1.2	6.7	16.9	19.9	22.7	18.1	3.4
3	α-Fenchene	953	951	–	–	–	0.1	–	–	1.0	–	0.1
4	Camphene	954	956	1.3	1.6	0.2	0.4	–	0.9	1.3	0.9	0.3
5	Thuja-2,4(10)-diene	960	957	0.8	–	–	–	–	–	–	–	–
6	Sabinene	975	975	–	–	–	0.3	–	0.3	–	–	–
7	*trans*-*p*-Menthane	979	976	–	0.4	–	–	–	–	1.1	1.1	–
8	Β-Pinene	979	980	19.7	1.6	0.3	1.7	2.0	1.2	2.0	1.2	–
9	p-3-Menthene	987	986	0.3	1.3	0.5	0.9	–	0.3	1.7	1.1	0.1
10	α-Phellandrene	1002	1005	1.8	2.1	13.5	2.2	13.0	6.4	3.5	15.0	8.9
11	δ-3-Carene	1011	1012	1.3	0.4	–	0.7	2.8	–	1.6	–	0.2
12	α-Terpinene	1017	1018	1.0	0.8	4.9	0.9	3.0	1.6	1.4	3.7	4.9
13	*p*-Cymene	1024	1028	5.1	47.3	35.2	25.5	41.2	21.9	32.7	37.7	59.1
14	β-Phellandrene	1029	1032	8.5	10.2	10.3	20.0	7.1	7.6	14.5	13.2	6.3
15	1,8-Cineole	1031	1034	–	–	4.8	2.6	4.4	1.8	–	3.6	2.5
16	Γ-Terpinene	1059	1060	0.5	–	0.5	0.3	–	0.3	–	–	–
17	Terpinolene	1088	1090	0.2	–	–	1.6	–	0.7	0.3	0.7	0.3
18	*p*-Cymenene	1089	1089	–	–	0.3	–	–	–	–	–	–
19	Linalool	1096	1099	0.3	–	–	–	–	–	–	–	–
20	endo-Fenchol	1116	1113	1.5	–	–	–	–	–	–	–	–
21	*trans*-Pinocarveol	1139	1140	0.9	–	–	–	–	–	–	–	–
22	*trans*-Dihydro-α-terpineol	1144	1145	13.7	4.8	3.4	–	–	2.6	4.9	1.5	–
23	Camphor	1146	1147	–	–	–	5.2	–	–	–	–	–
24	Borneol	1169	1168	1.6	–	0.3	0.7	–	–	–	–	–
25	Terpine-4-ol	1177	1177	0.9	–	0.9	2.0	–	0.4	–	–	0.4
26	Cymen-8-ol	1183	1187	–	–	–	6.6	–	–	–	–	–
27	α-Terpineol	1188	1190	4.5	1.0	6.4	8.2	–	2.6	1.3	–	1.4
28	Myrtenal	1195	1195	0.9	–	–	0.7	–	–	–	–	–
29	Methyl chavicol	1196	1197	3.0	–	–	–	–	–	–	–	–
30	Verbenone	1205	1208	0.8	–	–	0.6	–	–	–	–	–
31	*trans*-Piperitol	1208	1206	–	–	0.4	–	–	–	–	–	–
32	Cuminal	1242	1242	–	–	–	0.4	–	–	–	–	–
33	Carvone	1243	1246	–	–	–	0.7	–	–	–	–	–
34	Piperitone	1253	1256	–	–	–	0.4	–	–	–	–	0.5
35	Isobornyl acetate	1285	1285	0.9	–	–	–	–	–	–	–	–
36	α-Cubebene	1351	1351	–	0.4	–	–	–	0.3	0.5	–	0.6
37	Cyclosativene	1371	1366	–	–	0.3	–	–	0.6	–	–	–
38	α-Copaene	1376	1377	–	4.8	–	0.5	–	2.4	3.5	0.5	0.3
39	β-Elemene	1390	1390	–	–	–	–	–	0.4	–	–	–
40	Longifolene	1407	1401	3.3	–	–	–	–	–	–	–	–
41	β-Caryophyllene	1417	1417	0.9	2.0	0.6	1.1	–	2.3	2.0	–	–
42	*trans*-α-Bergamotene	1434	1434	–	–	0.2	0.2	–	–	–	–	–
43	α-Guaiene	1439	1437	–	–	0.4	–	–	0.6	–	–	–
44	6,9-Guaiadiene	1444	1442	–	–	0.4	–	–	0.6	–	–	–
45	*cis*-Muurola-3,5-diene	1448	1447	–	–	0.5	–	–	–	–	–	–
46	Spirolepechinene	1451	1447	–	–	–	–	–	0.8	–	–	–
47	α-Humulene	1454	1452	–	0.3	0.3	–	–	0.6	0.3	–	–
48	allo-Aromadendrene	1460	1461	–	–	–	–	–	–	–	–	0.5
49	*cis*-Muurola-4(14),5-diene	1466	1461	–	–	0.3	–	–	0.4	–	–	–
50	β-Acoradiene	1469	1472	–	–	0.8	–	–	–	–	–	–
51	10-epi-β-Acoradiene	1475	1472	–	–	–	–	–	0.9	–	–	–
52	γ-Muurolene	1479	1475	–	–	–	–	–	0.3	0.4	–	–
53	Germacrene D	1481	1478	–	–	–	–	–	0.8	0.4	–	–
54	γ-Himachalene	1481	1481	–	0.5	–	–	–	–	0.8	–	–
55	*cis*-β-Guaiene	1493	1489	–	–	0.3	–	–	–	–	–	–
56	Bicyclogermacrene	1500	1496	–	–	–	–	–	–	–	–	1.4
57	Epizonarene	1501	1495	–	–	0.3	–	–	0.6	–	–	–

(*Continued*)

Table 2. (*Continued*)

S.N	Compounds	RI^{lit}	RI^{*}	Percentage								
				White *breu*					Black *breu*			
				ALPM	AF	P10F	OPM	VPM	ALPM	AF	P10F	OPM
58	A-Bulnesene	1509	1502	–	–	0.5	–	–	0.7	–	–	–
59	δ-Amorphene	1512	1511	–	1.0	0.4	–	–	1.0	1.2	–	–
60	γ-Cadinene	1513	1513	–	–	6.1	–	–	10.3	–	–	–
61	δ-Cadinene	1523	1523	–	1.0	0.4	0.3	–	1.6	–	–	–
62	α-Cadinene	1538	1535	–	–	–	–	–	0.4	–	–	–
63	Spathulenol	1578	1578	–	–	0.3	–	–	–	–	–	1.1
64	Caryophyllene oxide	1583	1583	–	0.3	0.3	0.6	–	–	–	–	–
65	1,10-di-epi-Cubenol	1619	1611	–		1.2	–	–	1.4	–	–	–
Total identified (%)		–	–	96.6	99.6	97.1	92.5	90.8	96.1	99.1	98.7	92.3
Monoterpene hydrocarbons		–	–	62.7	83.5	66.5	59.8	86.4	60.7	83.5	92.4	83.3
Oxygenated monoterpenes		–	–	29.7	5.8	17.0	30.0	4.4	8.4	6.5	5.8	5.1
Sesquiterpene hydrocarbons		–	–	4.2	10.0	11.8	2.1	–	25.6	9.1	0.5	2.8
Oxygenated sesquiterpenes		–	–	–	0.3	1.8	0.6	–	1.4	–	–	1.1

Notes: Components are listed in order of their elution from HP-5MS column. RI^{lit}, linear retention indices from literature (15); LRI^{*}, linear retention indices in a HP-5 column. Percentage obtained by flame ionization detector (FID) peak area normalization.

was γ-cadinene (10.3%), present in the black *breu* from ALPM. Among these major constituents, both β-pinene and α-pinene have been reported as anticonvulsants (23), *p*-cymene (24) as anti-inflammatory and weakly antimicrobial, α-terpineol and α-pinene (25, 26) as possessing antimicrobial activity and α-pinene as exhibiting anti-inflammatory activity (27). Furthermore, it has been demonstrated that the chemical composition of *breu* resin essential oils may vary widely depending on both genetic and environmental features such as temperature, soil nutrient level, sun exposure and relative humidity. This variability is very common when the constituents, such as monoterpenes and sesquiterpenes, are volatile (7, 10, 18, 20). Because this work used raw materials from commercial sources, collected by different people from various places or times and stored under different conditions, the qualitative and quantitative variations found in the constitution of the essential oils are justifiable.

Physical characteristics of essential oils

The refractive index and optical rotation values obtained for each *breu* essential oil are reported in Table 1. The refractive index values ranged from 1.4769 (white *breu* from ALPM) to 1.4859 (white *breu* from P10F). In general, the samples of the white *breus* essential oils exhibited a greater refractive index than those of the black *breus*, except for the samples from ALPM and VPM markets, which showed distinctive organoleptic characteristics. These results may be related to a higher concentration of oxygenated compounds, such as aldehydes, alcohols, esters and oxides, in the white *breu* samples. A similar result was obtained for the optical rotation with values ranging from −14.15° (white *breu* from ALPM) to +29.96° (black *breu* from OPM). Again, with the exception of a sample from the VPM market, all of the values for the white *breu* essential oils were less than those of the black *breus*. These results can be explained by the presence of some oxygenated compounds in the white *breu* samples that are responsible for the levorotatory properties due to their chemical structures (28). In addition to presence of oxygenated compounds, the qualitative and quantitative constitution differences of each sample directly influence physicochemical parameters such as refractive index and optical rotation. Individually, each constituent will influence the speed and number of degrees at which they refract light and will affect the direction and degree to which light rays bend as they pass through the oil.

This is the first time that a comparative study of *breu* commercial samples has been described. This study is significant for demonstrating the absence of any standardization of the commercial samples. To enable the use of this raw material by pharmaceutical and cosmetic industries, it will be necessary to establish physical and chemical quality parameters with ranges of values for constituents and physicochemical characteristics.

Because the production of the oleoresin is related to the natural plant defense, extraction of these natural materials will be ethno-sustainable, avoiding predatory extraction that can lead to extinction. Because this is a widely available material and its main trading pattern in Amazon regions is related to boat caulk, the oleoresin has a low commercial value and its extraction is not in high demand. The discovery of new properties for *Protium* spp. oleoresins may help to generate a new

Table 3. Minimal inhibitory concentration (MIC) values of nine *breu* essential oils against selected fungi and bacteria.

	Microorganisms			
Sample	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
White *breu* ALPM	5 mg/mL	>5 mg/mL	2.5 mg/mL	>2.5 mg/mL
White *breu* AF	5 mg/mL	>5 mg/mL	>2.5 mg/mL	>2.5 mg/mL
White *breu* P10F	>5 mg/mL	>5 mg/mL	>2.5 mg/mL	>2.5 mg/mL
White *breu* OPM	>5 mg/mL	>5 mg/mL	>2.5 mg/mL	>2.5 mg/mL
White *breu* VPM	5 mg/mL	5 mg/mL	2.5 mg/mL	>2.5 mg/mL
Black *breu* ALPM	5 mg/mL	>5 mg/mL	2.5 mg/mL	>2.5 mg/mL
Black *breu* AF	5 mg/mL	>5 mg/mL	1.25 mg/mL	>2.5 mg/mL
Black *breu* P10F	>5 mg/mL	>5 mg/mL	>2.5 mg/mL	>2.5 mg/mL
Black *breu* OPM	>5 mg/mL	>5 mg/mL	>2.5 mg/mL	>2.5 mg/mL
Amphotericin B	–	–	0.19 μg/mL	0.25 μg/mL
Ciprofloxacin	0.25 μg/mL	0.03 μg/mL	–	–

source of income for many traditional communities of the Amazon.

Determination of antimicrobial activity

The MIC of each *breu* essential oil sample against selected microorganisms is presented in Table 3. In comparison with the positive controls, all of the essential oils exhibited low inhibitory activity, particularly against bacteria. All of the samples tested were more active against yeast-like and filamentous fungi, with MIC values equal or higher than 2.5 mg/mL. Bandeira et al. (8) observed that the essential oil from the oleoresin of *P. heptaphyllum* containing terpinolene (28.5%), limonene (16.9%), α-phellandrene (16.7%) and α-pinene (10.5%) was active against *C. albicans*, *S. aureus* and *E. coli*. These results suggest that the absence or low concentration of oxygenated monoterpenes and sesquiterpenes in the composition of these oils may be related to the low antimicrobial activity because the essential oils are primarily composed of mono-terpenes hydrocarbons (59.8–92.4%), which are slightly active or inactive in general (29). Some studies suggest that the antimicrobial activity of some essential oils is related to high concentrations of phenolic compounds, such as phenylpropanoids (eugenol, methyl eugenol and 4-ethyl-guaiacol) (30, 31) and phenolic monoterpenes (thymol and carvacrol) (29), which were not detected in the essential oils used in this study. Thus, the greatest inhibition found against *C. albicans* from the black *breu* from FA (MIC 1.25 mg/mL) may be related to the combination of all its constituents because no individual compound stands out when compared to the composition of other tested essential oils.

In vitro antimycobacterial activity evaluation

The antimycobacterial activity of the different *breu* essential oils samples was assayed in concentrations ranging from 3.5 to 100 μg/mL. *Mycobacterium tuberculosis* was slightly sensitive only to the black *breu* sample from ALPM at 100 μg/mL. This is the only essential oil that showed the presence of the sesquiterpene γ-cadinene (10.3%) among its major constituents. Although the essential oil of the white *breu* from F10P contained a moderate concentration of γ-cadinene (6.1%), no inhibition was demonstrated. This result may be associated with the low concentration of α-pinene (1.2%), as the black *breu* essential oil sample from ALPM contains a high concentration (19.9%) of this constituent. Thus, the combination of some constituents, such as γ-cadinene and α-pinene, a monoterpene with recognized antimicrobial activity (25, 26), may be responsible for the antimycobacterial activity of this essential oil. In a study conducted by Melliou et al. (32) of the antimicrobial activity of volatile constituents of different propolis samples from Greece, the essential oil containing α-pinene (18.2%) and γ-cadinene (3.7%) as two of its major constituents presented the greatest activity against some bacteria and fungi. The essential oil of *Salvia rubifolia* containing γ-cadinene and α-pinene as two of its major constituents also showed promising results against some species of bacteria (33).

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