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Original Article

Report on the Malungo expedition to the Erepecuru river, Oriximiná, Brazil. Part I: is there a difference between black and white *breu*?

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ABSTRACT

Species belonging to Burseraceae produce an oleoresin known in the north of Brazil as *breu*. They comprise an essential oil with a complex composition, and are used in Amazonia for smoking the environment, to caulk boats and for medicinal purposes. Depending on its organoleptic characteristics and on the *breu*-producing species, they are called white or black *breu*. In this work, we provide data about the *breu*-producing species occurring in the *quilombola* region of the Erepecuru river, the chemical composition, and whether it is possible to differentiate them based on their chemical composition and/or botanical identification. Aerial samples from *breu* trees and oleoresins were collected from 10 different individuals at 6 different sites on the Erepecuru river under the guidance of the *quilombolas*. Essential oils were extracted by hydrodistillation and characterized by GC–MS. From the analysis, 126 different substances were identified, with a large quantitative and qualitative variation. To better understand the chemical variations within the samples and to sort the variation into the categories of white or black *breu* as identified by the quilombola, we sorted the oil samples into five different sets according to their major compounds (A: δ-3-carene; B: *p*-cymene; C: γ-cadinene/*p*-cymene; D: limonene, β-phellandrene/α-terpineol; E: α-pinene/limonene). Essential oils from samples of white *breu* had the highest concentration of α-pinene, while a similarity in chemical composition could not be established for the black *breu* samples (sets A, B and C). Furthermore, a chemical similarity between a black *breu* (*Protium heptaphyllum* (Aubl.) Marchand) and a white *breu* (*Protium decandrum* (Aubl.) Marchand) sample was evidenced. In conclusion, it is difficult to establish definitions for white and black *breu* based on chemical, botanical or regional names. This designation is more cultural and regional than scientific and is based on the oleoresin production volume, its color aspect and scent.

Introduction

In December 2007, our research group at the Federal University of Rio de Janeiro obtained the first approval in Brazil to access traditional knowledge for bioprospecting purposes in *quilombola* communities of Oriximiná, Pará State, Brazil (Oliveira et al., 2010). Since then, we have been documenting the vast knowledge of the *quilombola* people from this region (Oliveira, 2009; Oliveira et al., 2011, 2012, 2015; Peçanha et al., 2013). By definition, "remnants of quilombos" or "quilombola" communities are ethnic groups with a specific historical background, specific territorial relations, and a presumption of black ancestry, that are related to resistance to oppression suffered historically (Oliveira et al., 2012). In the late 18th and early 19th centuries, the slaves that were used on cocoa, coffee, and cotton plantations as a labor force fled to remote areas, especially to regions of lakes and waterfalls that were difficult to access. Many expeditions were sent to destroy the *quilombos* and recapture the slaves, but some of them managed to escape by journeying up the Trombetas river along two routes: one along the course of the river Erepecurú (also known as Cuminá or Paru do Oeste) and the other toward the navigable stretches of the High Trombetas, after passing over waterfalls (Andrade, 1995; Acevedo and Castro, 1998). Many of these communities are still in full contact with the natural biodiversity of regions far from the urban area of Oriximiná. Their close contact with nature over centuries, the knowledge formed from an Indian–Black–Portuguese complex, and their geographic isolation, have brought to the members of these communities a vast knowledge of medicinal plants.

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Among the many plants and plant products used as remedies by this ethnic group, we were particularly interested in the *breu* oleoresins. In Brazil, the term *breu* is used to designate a resinous exudate, also known as *elemi*, produced by *Protium* species (Siani et al., 1999a). *Protium* is the main genus of the Burseraceae family, which includes eighteen genera with approximately 700 species, and is one of the most widespread in South America (Siani et al., 1999a; Weeks et al., 2005). Species belonging to this family produce fluid secretions (oleoresins) from bags and canals located in the bark or more deeply in the wood (Costa, 1994). These oleoresins are very aromatic, with economic, cultural and medicinal value, and can be blackened, whitish or even colorless (Ribeiro and Daly, 1999; Langenhein, 2003). A volatile oil can be obtained from this exudate by hydrodistillation. If recently produced, the oleoresin appears clear and plastic, but with oxidation and volatilization of some components it becomes hard, resinous, dark and brittle (Costa, 1994). 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* (Da Silva et al., 2013). They present a complex qualitative and quantitative chemical composition varying between the plant species of origin and the different environments in which they grow. Mono- and sesquiterpenes are the main volatile compounds which comprise the essential oil, while triterpenes are the major components of the resin (Carvalho et al., 2010; Hernández-Vázquez et al., 2010; Silva et al., 2009). Among these components, some volatiles have antimicrobial and antioxidant (Bandeira et al., 2006), analgesic (Rao et al., 2007), anti-inflammatory and antitumor (Siani et al., 1999a) activities. In addition, some of the resin triterpenes present anxiolytic and sedative (Aragão et al., 2006), anti-inflammatory (Medeiros et al., 2007) and analgesic (Pinto et al., 2008) activities.

According to Oliveira (2009), white *breu*, and less frequently black *breu*, oleoresins have been used by the quilombola of Oriximiná for the treatment of headaches by burning it and inhaling the smoke derived from its combustion, among other uses. In these communities, *breu* oleoresin is used either alone or in combination with *cumaru* seeds (*Dipteryx odorata* (Aubl.) Wild.) and with coffee powder or coffee grains. Depending on the visual appearance of the tree from which these oleoresins are collected and on their organoleptic characteristics, such as color and odor, the members of the local communities classify the *breu* trees as white or black.

In March 2012, we embarked on an expedition to the *quilombola* territories in the Erepecuru river in a search for different *breu* trees and oleoresins, as well as to try to determine what the *quilombolas* understand as white *breu*, black *breu*, and other *breu* denominations. In the literature, it has been stated that the criteria for the distinction between the different types of *breu* oleoresins are based only on their smell, without any identification of botanical differences among the species (Ramos et al., 2000). However, some species are directly related to the type of *breu* oleoresins. Examples include *Protium heptaphyllum* (Aubl.) Marchand, which is called a true white *breu* tree (Silva et al., 1977; Rodrigues, 1989; Siani et al., 1999b; Revilla, 2002; Da Matta, 2003; Silva, 2006b; Berg, 2010); *Protium spruceanum* Benth., also known as a white *breu* tree (Brandão, 2011); *Protium insigne* (Triana & Planch.) Engl., known as the *breu sucuruba* tree (Silva et al., 1977; Revilla, 2002); and *Tetragastris panamensis* (Engl.) Kuntze, called the black *breu* tree (Silva et al., 1977) or the white *breu* tree (Lima et al., 2001). Our journey to the Erepecuru river was named the "Malungo Expedition" after the bantu (an African dialect) term *Malungu* or *malungo*, which means, among other meanings, "companion", "brother", "from the same region", or "companion in suffering". In the kikongo language, it means canoe (Slenes, 1992). We felt that this was the perfect name for the expedition because of the ancestry of our *quilombola* companions, of all the suffering that was implied into taking a trip into the Amazonian forest and last, but not least, because we were traveling in canoes along the river. This region of the Erepecuru river, called the "Alto Erepecuru" (High Erepecuru), has been the site of many previous expeditions by foreign and Brazilian explorers, including Mme. Coudreau, between April and September 1900 (Coudreau, 1901), General Cândido Rondon and Gastão Cruls, between September 1928 and January 1929 (Cruls, 1973), and Ferreira d'Almeida, between July and November 1936 (Ferreira d'Almeida, 1937). Mme. Coudreau was the widow of Henri Anatole Coudreau, a French geographer who was hired by the Governor of the State of Pará to map the Amazon's tributaries in the 19th century. In 1899 he died in her arms from malarial fever on his expedition to the Trombetas river, but Mme. Coudreau continued the exploration work begun by her husband for the next seven years. In all these explorer's diaries there is mention of *breu* tree areas, but there is no register of the medicinal uses/indications of the breu oleoresins or a description of the scientific names of the species, except for the citation of *P. heptaphyllum* by Ferreira d'Almeida (1937). More recently, Acevedo and Castro (1998) noted the importance of the *breu* oleoresin commerce to the *quilombola* of Oriximiná, evidence of the importance of this product for these people.

In this study, we contribute to the knowledge of these oleoresins by providing data about the *breu* trees occurring in this region, the chemical composition of their oleoresins, and whether it is possible to differentiate white *breu* from black *breu* oleoresin based on its chemical composition and/or botanical identification of the tree of origin.

Experimental

Characterization of the search area

The municipality of Oriximiná, located in the State of Pará, northern Brazil, is bordered by Suriname, Guyana and French Guiana to the north, the cities of Faro, Juruti, and Óbidos to the south and east, and the States of Amazonas and Roraima to the west. It has an area of 107,603 km^2 and is the second largest municipality in the Brazilian territory. There are 33 known *quilombola* communities in Oriximiná that are divided into eight territories (Água Fria, BoaVista, Trombetas, Erepecuru, Alto Trombetas, Jamari/Último Quilombo, Moura, and Ariramba) that encompass more than 600,000 ha (Oliveira et al., 2015). The *quilombolas* are represented by their association, called the "Associação de Comunidades Remanescentes de Quilombos do Município de Oriximiná" or ARQMO (Remaining of the Quilombo Communities Association from Oriximiná City). We have been working with this group of *quilombola* since 2007 when we obtained the first authorization for access to the traditional knowledge associated with bioprospecting from the Directing Council of Genetic Heritage (Conselho de Gestão do Patrimônio Genético – CGEN) in Brazil, through Resolution no. 213 (06.12.2007) published in the Federal Official Gazette of Brazil on 27 December 2007.

The Malungo expedition, collection sites and ethnobotanical data collection

The Malungo expedition began on March 2, 2012 and ended on March 12th of the same year. The expedition began in Pancada and was composed of eleven people. Six were *quilombola* gatherers, members from the communities of Pancada, Jauari and Espirito Santo, specialized in the collection of Brazil nut and *breu* oleoresin, in a region known as Alto Erepecuru (High Erepecuru), an important conservation area of difficult access, where the only allowed activity is the extraction of non-timber resources. We used canoes to progress up the river because this part is non-navigable for larger

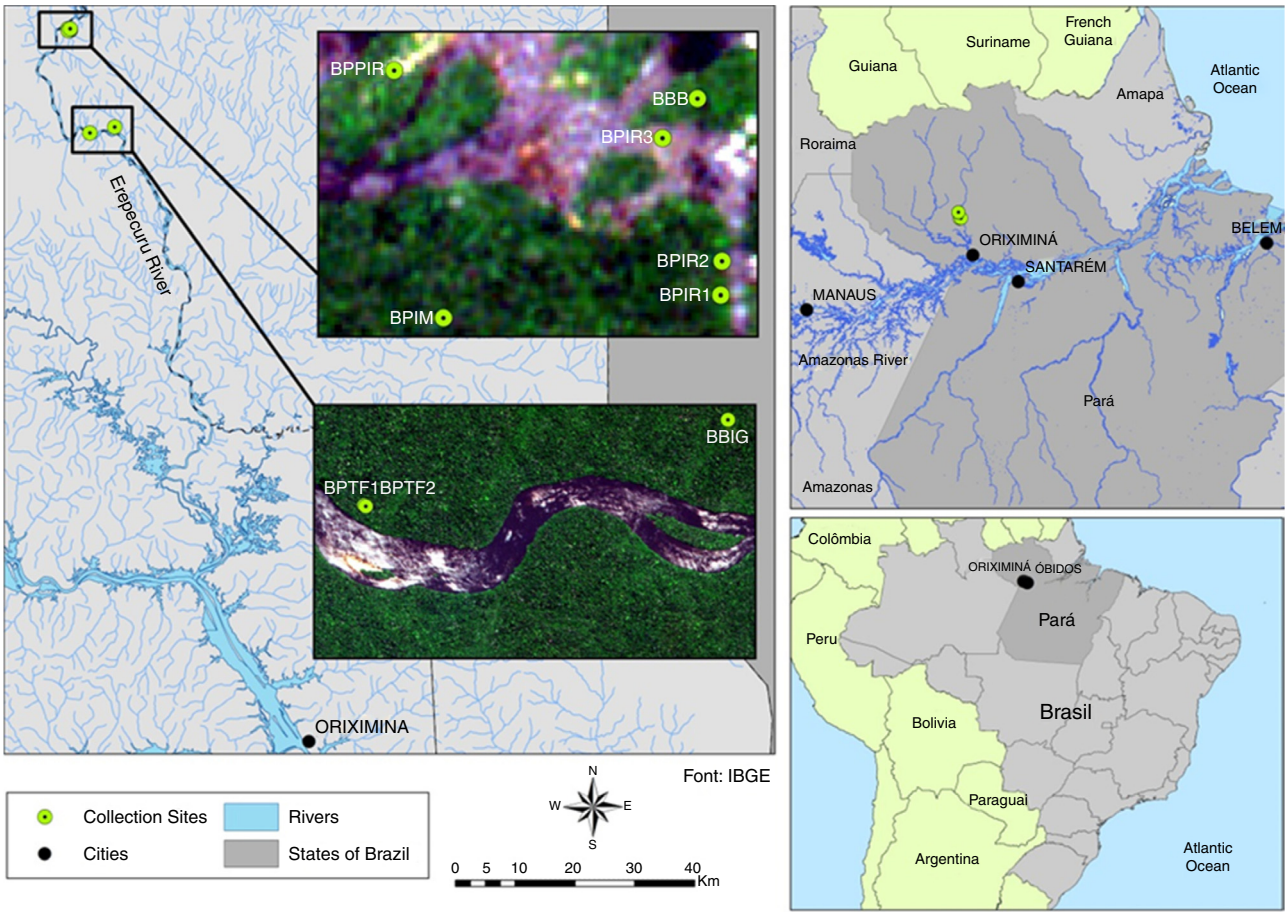

Fig. 1. Map representing the *quilombola* area of the Alto Erepecurú (High Erepecuru) region, Pará State, Brazil, with collection sites: BBIM – black *breu* Mel Island; BBPIR – black *breu* Cachoeira da Pirara; $BBIR_1$ – black *breu* Ilha do Recanto 1; $BBIR_2$ – black *breu* Ilha do Recanto 2; $BBIR_3$ – black *breu* Ilha do Recanto 3; $BBTF_1$ – black *breu* Terra Firme 1; $BBTF_2$ – black *breu* Terra Firme 2; WBB_1 – white *breu* Beliscão 1; WBB_2 – white *breu* Beliscão 2; WBIG – white *breu* Igarapé Grande.

boats because of rocks and rapids. The *quilombola* collecting areas for Brazil nuts and breu oleoresin are located in this region. We used participant observation and walk-in-the-woods with these local experts and stopped for camping and collecting *breu* trees and breu oleoresin samples in the sites indicated by the *quilombola* guides. The collection sites were selected based on the *quilombola* knowledge of black and white *breu* trees occurrence and are described in Figs. 1 and 2: Igarapé Grande (sample WBIG, S 00° 50,442′/W 56° 09,297′); Terra Firme (a *terra firme* landsite on the beach in front of the Praia Grande, samples $BBTF_1$, S 00° 50,971′/W 56° 11,548′ and $BBTF_2$, S 00° 50,978′/W 56° 11,538′); Beliscão (samples WBB_1 and WBB_2, no GPS signal, collection point estimated); Ilha do Recanto (samples $BBIR_1$, S 00° 41,729′/W 56° 13,287′, $BBIR_2$, S 00° 41,705′/W 56° 13,286′ and $BBIR_3$, S 00° 41,618′/W 56° 13,328′), Ilha do Mel (sample BBIM, no GPS signal, collection point estimated) and Cachoeira da Pirarara (sample BBPIR, S 00° 41,570′/W 56° 13,518′).

Sample collection and identification

Breu oleoresins (Fig. 3) and aerial samples (branches with leaves, with or without fruits) from different individual trees were collected between the 2nd and the 12th of March 2012. Oleoresins were dried in the sun for 30 min before being individually wrapped in aluminum foil. Aerial samples were wrapped in a sheet and soaked with alcohol 92° GL and then pressed to generate the voucher specimens. Species were identified by one of us (MFFM) and the vouchers were deposited at the INPA Herbarium, Manaus. The voucher numbers are given in Table 1.

Oleoresins moisture content analysis and essential oil extraction

Moisture content analysis was performed in triplicate for each sample collected. A 500 ml round bottomed flask connected to a Dean-Stark trap containing 5 g of oleoresin and 200 ml of toluene was heated to boiling for 2 h, then the volume of water was noted and the percentage (w/w) of moisture in the sample was calculated (AOCS, 1994). Oleoresins samples were fragmented and submitted to hydrodistillation for four hours using a Clevenger type apparatus. Essential oils were separated from the hydrolate by decantation, dried over anhydrous sodium sulfate and stored under refrigeration in sealed amber flasks.

Characterization and quantification of essential oils

Characterization of each essential oil was performed by gas chromatography coupled with mass spectrometry (GC–MS) using an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer. Separation was accomplished with a HP-5 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm phase thickness). Essential oils samples were dissolved in dichloromethane at a ratio of 1:1000 and a volume of 1 μl was injected. Operating conditions were as follows: split ratio 1:20; injector temperature 250 °C; carrier gas: helium, 1.0 ml/min, constant flow; column temperature, 60 °C (no hold), 3 °C per min to 240 °C; detector temperature: 280 °C. Mass spectra were acquired at 70 eV using a scan range of 40–450 *m/z* and a sampling rate of

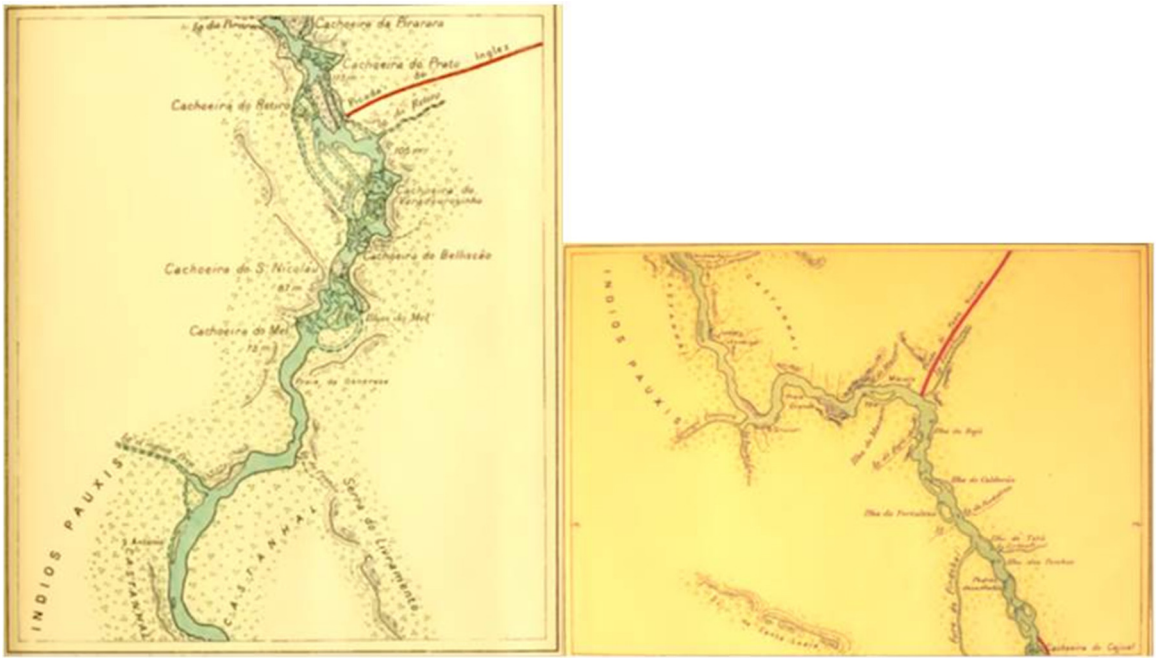

Fig. 2. Maps extracted from Mme. Coudreau's *Voyage au Cuminá* (Coudreau, 1901), showing collection sites. The names of the visited places are still the same.

3.15 scan/s. The ion source temperature was 230 °C, mass analyzer 150 °C and transfer line 260 °C.

Linear retention indices were calculated by injection of a series of *n*-alkanes (C_7–C_{26}) (Dool and Kratz, 1963) using de same column and condition as above for GC analyses. Identification of peaks was performed by comparison of mass spectra with an electronic library database (Wiley, 2000) and comparing their calculated linear retention indices with literature data (Adams, 2007).

Essential oils relative compositions were obtained using gas chromatography coupled with flame ionization detection (GC-FID). Analyses were performed using an Agilent 7890A gas chromatograph and separation was accomplished with a HP-5 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm phase thickness). The injection procedure and conditions was the same as described above, except the carrier gas, which was hydrogen, 1.5 ml/min.

Aiming the separation of co-eluted peaks and a more accurate identification of some major components, the essential oil samples and the authentic standards δ-3-carene, *p*-cymene and limonene were analyzed by GC–MS using an INNOWAX polyethylene glycol polar column (25 m × 0.2 mm i.d.). The injection procedure and conditions were the same as described above and the carrier gas was helium at a flow of 1 ml/min.

Essential oil physicochemical characterization

The optical rotation was determined using a Perking Elmer 341 digital polarimeter. Analyzes were performed in a thermostated 10 mm cell at 20 °C and with monochromatic 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. Both assays were performed in triplicate.

Results and discussion

Collection sites and identification of collected species

The *breu* oleoresin collection sites are depicted in maps shown in Figures 1 and 2. Figure 2 is a reproduction of parts of the maps produced by Mme. Coudreau (1901) referring to the sites where samples were collected. They are reproduced here (no copyrights) because they reliably show the names of the places where the plants were collected, which are the same today (Praia Grande, Ilha do Mel, Cachoeira do Mel, Cachoeira da Pirarara, Beliscão).

Figure 3 describes the aspects of the oleoresins at the moment they were collected. The differentiation between white and black *breu* oleoresin in the tree stem is very difficult because both types

Table 1
Sample codes, *breu* trees designation (white or black) given by the *quilombola*, voucher numbers, and identified species for each collected sample.

Sample	Voucher numbers	Popular name	Identified species
BBIM	265857	Black *breu* or *breuzinho*	*Protium heptaphyllum* (Aubl.) Marchand
BBPIR	265850	Black *breu*	*Protium decandrum* (Aubl.) Marchand
$BBIR_1$	265852	Black *breu*	*Protium heptaphyllum* (Aubl.) Marchand
$BBIR_2$	265851	Black *breu*	*Protium heptaphyllum (Aubl.) Marchand*
$BBIR_3$	265855	Black *breu*	*Protium heptaphyllum* (Aubl.) Marchand
WBB_1	265856	White *breu*	*Protium decandrum (Aubl.) Marchand*
$BBTF_1$	265859	Black *breu* or *sucuruba*	*Protium cf. opacum Swart*
$BBTF_2$	265858	Black *breu* or *sucuruba*	*Protium altsonii Sandwith*
WBB_2	265954	White *breu*	*Protium occultum D.C. Daly*
WBIG	265853	White *breu*	*Protium strumosum* Daly

BBIM, black breu Ilha do Mel; BBPIR, black breu Cachoeira da Pirara; $BBIR_1$, black breu Ilha do Recanto 1; $BBIR_2$, black breu Ilha do Recanto 2; $BBIR_3$, black breu Ilha do Recanto 3; WBB_1, white breu Beliscão 1; $BBTF_1$, black breu Terra Firme 1; $BBTF_2$, black breu Terra Firme 2; WBB_2, white breu Beliscão 2; WBIG, white breu Igarapé Grande.

Fig. 3. General aspect of the different *Breu* samples in the trees: (A) BBIM – black *breu* Mel Island; (B) BBPIR – black *breu* Cachoeira da Pirara; (C) $BBIR_1$ – black *breu* Ilha do Recanto 1; (D) $BBIR_2$ – black *breu* Ilha do Recanto 2; (E) $BBIR_3$ – black *breu* Ilha do Recanto 3; (F) WBB_1 – white *breu* Beliscão 1; (G) $BBTF_1$ – black *breu* Terra Firme 1; (H) $BBTF_2$ – black *breu* Terra Firme 2; (I) WBB_2 – white *breu* Beliscão 2; (J) WBIG – white *breu* Igarapé Grande.

have very similar coloring, with whitish and/or darkened parts. Other organoleptic characteristics of the oleoresin, such as texture and fragrance, do not guarantee the distinction between black and white *breu* oleoresins. Both can vary with the exposure time on the stalk and loss of the more volatile components. Furthermore, the chemical composition influences the oleoresin fragrance which, despite being quite similar, is of different intensities. It is interesting to note that the species *P. heptaphyllum* is often described in

the literature as a white *breu* oleoresin-producing species (Silva et al., 1977; Siani et al., 1999b), whilst in the present work it was characterized by the *quilombola* people as a black *breu* oleoresin-producing tree.

Table 1 displays the *breu* tree designations (white or black) given by the *quilombolas* and the corresponding scientific names of each collected sample. All identified species belong to the genus *Protium*.

According to the *quilombola* reports, white *breu* oleoresin is more fragrant, is produced in less quantity, and is used in the treatment of some diseases, such as headaches, while black *breu* oleoresin is mostly used to repair canoes and to smoke the environment. In addition to these differences, the black *breu* trees were further differentiated by the quilombola into *breuzinho* – short trees with a slender stalk; and *breu sucuruba* – tall trees with a thick stalk located away from the river margins. According to the quilombolas, white breu-producing trees are also tall and found away from the river, but have thinner stalks.

Characteristics of essential oils and chemical composition

The essential oils had the characteristic fragrance of *breu* oleoresin. They appeared clear and colorless at the beginning of the extraction, turning to a slightly yellowish tinge at the end, except for the essential oil from the sample $BBIR_2$, which became greenish, probably due to the presence of a chamazulene precursor.

The moisture content in each oleoresin sample and their essential oil yields (dry basis) are shown in Table 2

. The WBIG oleoresin showed the best yield (5.4%, w/w), while the lowest yield was for WBB_2 (0.8%, w/w). These results demonstrate that the amount of essential oil is not only related to the type of *breu* oleoresin – white or black – but also to environmental conditions such as temperature and humidity, as well as to exposure time in the stem and chemical composition, which will directly influence the degree of volatilization and oxidation (Costa, 1994; Ramos et al., 2000; Silva, 2006a).

The primary compounds with relative % peak area above 0.02 identified in the 10 essential oils obtained from each *breu* oleoresin sample are shown in Table 2. A total of 126 substances were identified, showing the complexity of their chemical composition, as well as the large quantitative and qualitative variation in their components. All the essential oils had a high percentage of identified components, with the exception of the sample $BBIR_3$ (62.8% of identified components).

After evaluation of the mass spectra and retention indexes of δ-3-carene, *p*-cymene and limonene eluted in an HP-5 (apolar) column, doubts about their presence and/or co-elution were raised. Therefore, essential oils and authentic standards were analyzed by GC–MS with a polyethylene glycol polar column to resolve the co-eluted compounds. Peaks of δ-3-carene, limonene and *p*-cymene in the polar column showed retention times and retention indices close to those reported for this column (Davies, 1990). In this type of column, a small change in programming can profoundly affect the retention index, leading to variations sometimes close to 50 units (Davies, 1990). In the polar column, the δ-3-carene and iso-sylvestrene peaks were separated, as well as the limonene and β-phellandrene mixture, present in all essential oils. In addition, the polar column analysis helped to confirm the presence of other major compounds such as *p*-cymene, α-pinene and γ-cadinene (Supplementary material).

Despite the complexity and large variation between the chemical composition of the different *breu* essential oils, many substances are common to all the samples and appear in varying concentrations. The monoterpene hydrocarbons α-pinene, limonene, β-phellandrene, *p*-cymene and the monoterpene alcohol α-terpineol are the only components present in all samples. Other very common monoterpenes include α-thujene, camphene, α-phellandrene, δ-3-carene, α-terpinene and terpinolene. Oxygenated monoterpenes and sesquiterpenes are also present in the essential oils, but with a more heterogeneous distribution. The chemical composition of all essential oils is compatible with literature data describing the composition of essential oils from oleoresins of different species of *Protium* (Ramos et al., 2000; Siani et al., 2004; Silva, 2006a; Tafurt-García and Muñoz-Acevedo, 2012; Da Silva et al., 2013).

In an attempt to better understand the chemical variation within the essential oil compositions and to sort this parameter by the definitions of white or black *breu* oleoresins using the *quilombola* system, we sorted the oil samples into five different sets, A–E.

Set A contained four samples of essential oils from black *breu* oleoresins – BBIM, BBPIR, $BBIR_1$ and $BBIR_2$ – originating from *P. heptaphyllum* and *Protium decandrum*. In this set, δ-3-carene and *iso*-sylvestrene were the major components, in mixtures varying from 40.9% to 79.5% (Table 2). Calculating the proportions between these two components in each of the essential oils after chromatographic analysis with a polar column revealed that δ-3-carene was always the major component. This is a new observation; other studies of *P. heptaphyllum* and varieties do not report this monoterpene as the major compound in their essential oils (Marques et al., 2010; Tafurt-García and Muñoz-Acevedo, 2012). Furthermore, according to Carvalho et al. (2010), the *P. decandrum* resin essential oil contains a higher concentration of sesquiterpene hydrocarbons and *trans*-α-bergamotene as the major components. As well as containing δ-3-carene and *iso*-sylvestrene as the major compounds in common, these essential oils showed a very similar composition, with a percentage of monoterpenes above 88% (Table 2).

The essential oils of $BBIR_3$ and WBB_1 contained *p*-cymene as their major component and a chemical composition rich in monoterpene hydrocarbons (Table 2); therefore, they were placed in set B. *p*-Cymene is the monoterpene most commonly described as the major compound in essential oils from *Protium* oleoresins (Ramos et al., 2000; Siani et al., 2004; Silva, 2006a; Tafurt-García and Muñoz-Acevedo, 2012; Da Silva et al., 2013). It is interesting that sample $BBIR_3$ corresponds to the oil obtained from the oleoresin of a black *breu* tree, *P. heptaphyllum*, while WBB_1 corresponds to that obtained from a white *breu* tree sample, *P. decandrum*. In addition, it is important to note that oleoresins obtained from trees of the same species (*P. decandrum*) can have different chemical compositions and be classified as black (BBPIR) or white (WBB_1) *breu*.

The samples collected from the banks of the river, $BBTF_1$ (*Protium opacum*) and $BBTF_2$ (*Protium altsonii*) showed a similar chemical composition, although they came from trees belonging to different species, showing the influence of environment in the composition of oleoresins. Although not having the same major component – γ-cadinene (14.4%) in $BBTF_1$ and *p*-cymene (16.4%) in $BBTF_2$ – both oils were placed in set C because they are the only ones with more sesquiterpenes than monoterpenes. In addition, both showed a high concentration of γ-cadinene (14.4% in $BBTF_1$ and 9.5% in $BBTF_2$), agreeing with data from previous studies (Da Silva et al., 2013). The $BBTF_2$ compositional data differ from those presented by Ramos et al. (2000) for the *P. altsonii* oleoresin essential oil, in which the monoterpene hydrocarbon concentration is higher and the major components are α-pinene, *p*-menth-3-ene and *p*-cymene. Curiously, both *breu* trees were classified as black *breu* or "breu sucuruba" by the quilombolas. Coincidentally, these were the only trees with completely black oleoresins at the time of collection.

Essential oil from oleoresins of WBB_2, identified as *Protium occultum* (Daly), contained the monoterpene hydrocarbons limonene and β-phellandrene (41.1%) and the oxygenated monoterpene α-terpineol (30.9%) as the major compounds. In addition, this was the only oil with a high content of oxygenated

Table 2
Breu Oleoresin moisture content and essential oils yields, physicochemical properties, chemical compositions and their corresponding grouping into sets A–B according to their major components.

		BBIM	BBPIR	BBIR1	BBIR2	BBIR3	WBB1	BBTF1	BBTF2	WBB2	WBIG
Moisture %		4	6	7	14	6	10	9	2	9	12
Yield % (w/w)		1.2	3.86	1.79	2.47	2.9	2.85	1.02	2.59	0.79	5.36
Refractive index[a]		1.4733	1.4693	1.4720	1.4693	1.4703	1.4730	1.4937	1.4830	1.4730	1.4673
Optical rotation (°)[a]		−0.059	+3.485	−0.282	+1.507	+3.664	+2.321	−0.668	+0.733	−2.179	+2.510
Essential oil grouping according to major compounds			A				B		C	D	E
S.N.	Substance	IR_{lit}**	IR***	Percentage (%)							
1	α-thujene	930	927	–	1.2	–	0.4	0.3	–	0.1	0.2	–	0.9
2	α-pinene	939	934	2.0	4.9	2.2	1.7	1.2	19.0	0.2	0.9	8.0	57.7
3	camphene	954	949	–	2.2	0.4	–	–	0.3	–	0.3	–	1.2
4	verbenene	967	969	1.5	–	0.8	0.4	–	–	–	–	–	–
5	sabinene	975	973	–	–	–	–	–	0.2	–	–	–	–
6	*trans*-*p*-menthane	979	975	–	–	–	–	–	–	0.1	–	1.0	–
7	β-pinene	979	980	–	2.3	0.5	–	–	3.6	–	–	–	9.3
8	2-menthene	-	980	0.6	–	–	0.6	–	–	0.5	0.8	–	–
9	3-*p*-menthene	987	984	–	–	–	–	–	–	0.7	1.1	–	0.9
10	mircene	990	990	0.5	1.7	0.3	0.6	–	–	–	–	–	–
11	camphane	–	1001	–	–	–	0.7	0.7	–	–	–	–	–
12	bornane	–	1001	–	–	–	–	–	3.0	–	–	–	–
13	1,3,8-*p*-menthatriene	–	1008	–	–	–	–	0.5	–	–	–	–	–
14	α-phellandrene	1002	1005	0.9	–	2.1	12.9	–	21.0	4.0	–	–	–
15	mix (δ-3-carene and *iso*-sylvestrene)	1011	1011	69.0	40.9	79.5	56.4	14.7	0.8	–	1.2	0.5	–
16	α-terpinene	1017	1017	–	–	–	1.6	–	5.5	2.7	–	–	0.6
17	*p*-cymene	1024	1026	6.4	13.4	3.5	14.0	33.0	32.4	6.6	16.3	10.4	9.2
18	1-*p*-menthene	1026	1022	–	–	–	–	–	0.7	–	0.2	0.8	–
19	mix (limonene and β-phellandrene)	1029	1028	5.7	20.3	3.2	6.8	–	12.0	4.3	–	41.1	10.8
20	1,8-cineole	1031	1031	–	–	1.5	–	–	–	–	–	–	–
21	γ-terpinene	1059	1059	–	1.6	–	–	–	0.5	0.2	–	–	3.4
22	*m*-cyminene	1085	1085	–	–	–	–	0.2	–	–	–	–	–
23	terpinolene	1088	1088	1.1	1.4	0.7	0.7	–	–	1.0	–	0.7	0.5
24	*p*-cymenene	1091	1092	–	–	–	–	0.6	–	–	0.1	–	–
25	linalool	1096	1102	–	0.4	–	–	–	–	–	–	–	–
26	*cis*-*p*-menth-2en-1-ol	1121	1123	–	–	–	–	0.2	–	–	0.1	–	–
27	cyclooctanone	–	1129	–	–	–	–	0.5	–	–	0.5	–	–
28	*cis*-limonene oxide	1136	1137	–	–	–	–	0.4	–	–	–	–	–
29	*trans*-dihydro-β-terpineol	1138	1137	–	–	–	–	–	–	0.1	–	–	–
30	camphor	1146	1146	–	0.5	0.8	–	–	0.5	–	–	–	1.4
31	*trans*-dihydro-α-terpineol	1147	1147	–	–	–	–	1.0	–	2.3	2.4	3.3	–
32	*cis*-dihydro-β-terpineol	1160	1158	–	–	–	–	–	–	0.04	–	–	–
33	*cis*-dihydro-α-terpineol	1164	1162	–	–	–	–	–	–	0.1	0.2	–	–
34	*p*-mentha-1,5-dien-8-ol	1170	1167	2.4	0.4	1.4	–	–	–	–	–	–	–
35	borneol	1169	1174	–	–	–	–	0.2	–	–	0.2	–	–
36	terpinen-4-ol	1177	1178	–	0.2	–	–	–	–	0.1	0.2	–	0.2
37	*p*-cymen-8-ol	1179	1182	2.7	0.4	–	–	–	–	–	–	–	–
38	criptone	1185	1189	–	–	–	–	–	–	–	1.1	–	–
39	α-terpineol	1188	1191	3.3	0.7	1.6	0.7	2.3	0.5	0.7	0.5	30.9	2.2
40	phellandrene epoxide	–	1205	–	0.4	–	–	–	–	–	0.2	–	–
41	γ-terpineol	1199	1199	–	–	–	–	–	–	0.1	–	2.1	–
42	verbenone	1206	1209	0.6	0.1	0.2	–	0.6	–	–	–	–	–
43	*(E)*-ocimenone	1238	1239	–	–	–	–	0.4	–	–	–	–	–
44	cuminal	1241	1241	–	–	–	–	0.6	–	–	0.3	–	–
45	carvotanacetone	1247	1250	–	–	–	–	0.3	–	–	–	–	–
46	piperitone	1252	1255	–	0.4	–	–	–	–	–	–	–	–
47	*trans*-ascaridol glycol	1269	1273	–	–	–	–	0.7	–	–	0.2	–	–
48	carvacrol	1299	1305	–	0.1	–	–	–	–	–	–	–	–
49	*p*-vinyl-guaiacol	1309	1312	–	0.2	–	–	–	–	–	–	–	–
50	δ-elemene	1338	1339	–	–	–	–	–	–	0.1	–	–	–
51	α-cubebene	1351	1351	–	0.1	–	–	0.5	–	3.1	3.9	–	–
52	cyclosativene	1371	1370	–	–	–	–	–	–	1.5	1.3	–	–
53	α-ylangene	1375	1373	–	0.2	–	–	–	–	0.4	0.2	–	–
54	α-copaene	1376	1377	–	–	–	–	0.6	–	0.8	1.1	–	–
55	β-cubebene	1388	1391	0.5	0.1	–	–	–	–	0.5	0.5	–	–
56	*iso*-longifolene	1390	1389	–	–	–	–	–	–	0.3	0.3	–	–
57	β-elemene	1390	1393	–	0.1	–	–	–	–	–	0.3	–	–
58	cyperene	1398	1398	–	–	–	–	–	–	0.6	2.3	–	–
59	α-cedrene	1411	1414	–	–	–	–	–	–	1.7	1.6	–	–
60	α-*cis*-bergamotene	1412	1416	–	–	–	–	–	–	–	–	–	0.5
61	β-caryophyllene	1419	1423	–	–	–	–	–	–	2.7	–	–	–
62	β-cedrene	1420	1420	–	0.3	–	–	–	–	–	2.7	–	–
63	*cis*-thujopsene	1431	1433	–	–	–	–	–	–	0.7	0.5	–	–
64	*trans*-6-hydroxy-*p*-menth-1-en-3-one	–	1435	–	–	–	–	–	–	–	0.2	–	–
65	β-copaene	1432	1430	–	0.1	–	–	–	–	–	–	–	–
66	*trans*-α-bergamotene	1434	1437	–	0.1	–	–	–	–	3.2	2.6	–	–
67	β-humulene	1438	1440	–	0.02	–	–	0.2	–	–	–	–	–

Table 2 (*Continued*)

S.N.	Substance	IR_{lit}**	IR***	Percentage (%)									
68	α-guaiene	1439	1444	–	–	–	–	0.3	–	1.6	–	–	–
69	aromadendrene	1441	1448	–	–	–	–	–	–	2.0	–	–	–
70	β-barbatene	1442	1445	–	–	–	–	–	–	–	2.3	–	–
71	6,9-guaiadiene	1444	1444	–	0.3	–	0.5	–	–	–	–	–	–
72	*cis*-muurola-3,5-diene	1450	1450	–	0.1	–	–	–	–	–	–	–	–
73	α-*neo*-clovene	1454	1455	–	–	–	–	–	–	5.3	–	–	–
74	α-humulene	1454	1454	–	0.03	–	–	–	–	–	–	–	–
75	khusimene	1455	1454	–	–	–	–	–	–	–	2.9	–	–
76	sesquisabinene	1459	1461	–	–	–	–	–	–	1.0	1.0	–	–
77	*allo*-aromadendrene	1460	1461	–	0.03	–	–	–	–	–	–	–	–
78	*cis*-cadina-1(6),4-diene	1463	1465	–	–	–	–	–	–	1.8	0.2	–	–
79	α-acoradiene	1466	1469	–	–	–	–	–	–	–	0.5	–	–
80	α-neocallitropsene	1476	1481	–	–	–	–	–	–	7.3	–	–	–
81	γ-gurjunene	1477	1480	–	–	–	–	–	–	–	5.2	–	–
82	γ-muurolene	1479	1478	–	0.1	–	–	0.2	–	0.8	–	–	–
83	α-curcumene	1480	1486	–	–	–	–	–	–	–	0.6	–	–
84	germacrene D	1481	1482	–	0.8	–	–	–	–	–	–	–	–
85	α-amorphene	1484	1490	–	0.1	–	–	–	–	–	–	–	–
86	*trans*-muurola-4(14), 5-diene	1490	1483	–	–	–	–	–	–	–	0.2	–	–
87	*cis*-β-guaiene	1493	1496	–	–	–	–	–	–	2.3	–	–	–
88	*epi*-cubebol	1494	1497	–	–	–	–	–	–	–	0.2	–	–
89	valencene	1496	1503	–	–	–	–	–	–	1.7	–	–	–
90	α-selinene	1498	1501	–	–	–	–	–	–	–	0.3	–	–
91	*trans*-β-guaiene	1502	1513	–	–	–	–	–	–	4.9	–	–	–
92	cuparene	1504	1509	–	–	–	–	–	–	–	1.8	–	–
93	δ-amorphene	1512	1508	–	0.3	–	–	–	–	–	–	–	–
94	γ-cadinene	1513	1515	0.5	0.1	–	–	0.1	–	14.4	9.5	–	–
95	*trans*-calamenene	1522	1525	–	–	–	–	0.4	–	–	–	–	–
96	δ-cadinene	1523	1525	–	0.1	–	–	–	–	4.4	4.3	–	–
97	kessane	1530	1533	–	–	–	–	–	–	–	0.4	–	–
98	(*E*)-γ-bisabolene	1531	1533	–	–	–	–	–	–	–	–	–	1.1
99	*trans*-cadina-1,4-diene	1534	1541	–	–	–	–	–	–	3.6	–	–	–
100	α-cadinene	1538	1541	–	–		–	–	–	0.9	0.5	–	–
101	α-calacorene	1545	1546	–	0.04	–	–	–	–	0.9	0.7	–	–
102	timohydroquinone	1555	1565	–	–	–	–	0.1	–	–	–	–	–
103	germacrene B	1561	1569	–	–	–	–	–	–	0.6	–	–	–
104	β-calacorene	1565	1567	–	–	–	–	–	–	–	0.4	–	–
105	caryophyllene oxide	1583	1585	–	–	–	–	0.2	–	0.4	3.3	–	–
106	salvial-4(14)-en-1-one	1594	1595	–	0.03	–	–	0.1	–	–	–	–	–
107	rosifoliol	1600	1598	–	–	–	–	–	–	0.2	–	–	–
108	5-*epi*-7-epi-α-eudesmol	1607	1611	–	–	–	–	–	–	0.1	–	–	–
109	humulene epoxide II	1608	1613	–	–	–	–	–	–	–	0.7	–	–
110	junenol	1619	1620	–	0.1	–	–	0.6	–	–	–	–	–
111	1,10-di-*epi*-cubenol	1619	1620	–	–	–	–	–	–	3.5	2.4	–	–
112	dillapiol	1620	1627	–	0.1	–	–	–	–	–	–	–	–
113	1-*epi*-cubenol	1628	1632	–	–	–	–	–	–	0.1	–	–	–
114	*epi*-α-cadinol	1640	1642	1.2	0.1	–	–	0.1	–	0.3	0.2	–	–
115	*cis*-calamenen-10-ol	1661	1663	–	–	–	–	–	–	–	0.4	–	–
116	*trans*-calamenen-10-ol	1669	1672	–	–	–	–	–	–	–	0.3	–	–
117	bulnesol	1671	1671	–	–	–	–	–	–	0.2	–	–	–
118	cadalene	1676	1677	–	–	–	–	–	–	0.1	0.4	–	–
119	α-bisabolol	1685	1686	–	–	–	–	–	–	0.2	–	–	–
120	5-*neo*-cedranol	1685	1687	–	–	–	–	–	–	–	0.2	–	–
121	eudesma-4(15),7-dien-1β-ol	1688	1689	–	–	–	–	0.1	–	–	–	–	–
122	*cis*-14-*nor*-muurol-5-en-4-one	1689	1691	–	–	–	–	–	–	–	0.5	–	–
123	davanol acetate	1689	1695	–	–	–	–	–	–	–	0.2	–	–
124	10-*nor*-calamenen-10-one	1702	1705	–	–	–	–	–	–	–	0.3	–	–
	Total identified components (%)	–	–	99.0	97.0	98.7	98.0	61.9	100	98.0	84.5	98.8	99.9
	Monoterpenes hydrocarbons	–	–	87.7	89.9	93.2	96.8	51.2	99.0	20.4	21.1	62.5	94.5
	Oxigenated monoterpenes	–	–	9.0	3.8	5.5	0.7	7.2	1.0	3.4	6.0	36.3	3.8
	Sesquiterpenes hydrocarbons	–	–	1.0	3.0	–	0.5	2.3	–	69.1	48.1	–	1.6
	Oxygenated sesquiterpenes	–	–	1.2	0.3	–	–	1.2	–	5.1	9.3	–	–

Components listed in order of elution of the HP-5 column.
IR_{lit}**, retention indices obtained in the literature; IR*** linear retention indices calculated from a homologous series of n-alkanes C_7–C_{26}.
Percentage obtained by normalizing the FID peaks area.
BBIM, black breu Ilha do Mel; BBPIR, black breu Cachoeira da Pirara; $BBIR_1$, black breu Ilha do Recanto 1; $BBIR_2$, black breu Ilha do Recanto 2; $BBIR_3$, black breu Ilha do Recanto 3; WBB_1, white breu Beliscão 1; $BBTF_1$, black breu Terra Firme 1; $BBTF_2$, black breu Terra Firme 2; WBB_2, white breu Beliscão 2; WBIG, white breu Igarapé Grande.
[a] Measured at 20 °C.

terpenes (36.3%) (Table 2). We named this sample as set D. Again, according to chromatographic analysis with a polar column, there was a higher proportion of limonene compared to β-phellandrene. Limonene has already been identified as the major component (23.2%) in essential oils of Burseraceae oleoresins, such as in *Bursera graveolens* Kunth. (Muñoz-Acevedo et al., 2013), similar to our results. High concentrations of these two monoterpene hydrocarbons were also found in oleoresin samples from *Protium strumosum*,

while concentrations of β-phellandrene close to 40% were found in oleoresin samples from *Protium nitidifolium* (Ramos et al., 2000). However, no data for the chemical characterization of the oleoresin essential oil from *P. occultum* were found in the literature.

The oleoresin from sample WBIG, identified as *P. strumosum* (Daly), provided an essential oil rich in monoterpene hydrocarbons (94.5%) containing α-pinene (57.7%) and limonene (10.8%) as the major components (Table 2). Therefore, this sample was classified as set E. Some other *breu* essential oils also contained α-pinene as the major compound (Ramos et al., 2000; Silva, 2006a). In contrast, a sample of the essential oil from an oleoresin of *P. strumosum* studied by Ramos et al. (2000) revealed *p*-cymene (27.4%), terpinolene (22.4%) and β-phellandrene (17.4%) as the major components, and only 1.9% of α-pinene, which is very different from our results. The presence of limonene in the range of 10% and a high concentration of α-pinene was reported by Tafurt-García and Muñoz-Acevedo (2012) for the essential oil of the oleoresin from *P. heptaphyllum* (Aubl.) March.

It is important to note that the trees with essential oils with the highest concentrations of α-pinene – WBB_1, *P. decandrum*; WBB_2, *P. occultum*; and WBIG, *P. strumosum* – were identified by local quilombolas as white *breu* trees. The white *breu* oleoresin is mainly used by them as a medicine for headaches. It is possible that α-pinene, a monoterpene with anti-inflammatory (Sá et al., 2013) and antinociceptive (Quintão et al., 2010) activities, is involved with this therapeutic action. However, this is the only chemical similarity found in the samples classified by the *quilombola* as white *breu* oleoresin. Their major compounds, as well as their mono- and sesquiterpene composition (oxygenated or not), are quite different. Such chemical lack of uniformity also occurs between samples designated by the quilombolas as black *breu* oleoresins. Furthermore, a similarity between the chemical composition of the essential oil from a black *breu* oleoresin sample ($BBIR_3$) and a white *breu* oleoresin sample (WBB_1) was evidenced, such that they were classified in the same set (B). These results demonstrate that the analysis of the chemical composition alone does not differentiate between white and black *breu* trees or oleoresins.

In addition, environmental factors such as soil, moisture and temperature can also influence the type of oleoresin produced by the tree (Siani et al., 1999b, 2004; Marques et al., 2010; Silva, 2006a). The amount of oleoresin produced is related to the degree of injury in the stem of the tree. When exposed to natural elements, this oleoresin may suffer losses of the essential oil components by volatilization, becoming harder and more brittle. Similarly, the oxidation of some of its components can generate products, which, in combination with environmental dirt, leave this oleoresin darker in some parts. These natural organoleptic changes can lead to confusion in the differentiation between white and black oleoresins, which explains in part the controversial designations.

Essential oil physicochemical characterization

Refractive indices and optical rotation values for each essential oil are presented in Table 2. The refractive index values ranged from 1.4673 (WBIG) to 1.4937 ($BBTF_1$). This result is in agreement with those in the literature for the essential oils from *Protium* oleoresins (Ramos et al., 2000; Silva, 2006a; Da Silva et al., 2013). Likewise, the values for optical rotation, which varied from −0.668° ($BBTF_1$) to +3.664° ($BBIR_3$), are also consistent with the literature data (Da Silva et al., 2013).

The refractive index of *breu* essential oils is high, always higher than the index for water (1.3330), and within the range of 1.4, varying at the second decimal place. This is a constant for these essential oils; a variation at the first decimal place may represent an alteration in or tampering with the essential oil. The variation found in the refractive indices is associated with the qualitative and quantitative chemical composition of essential oils because each of the components individually influences the speed and the angle at which the light is refracted. In the same way, these components influence the direction and the degree of deviation that the ray of polarized light undergoes while crossing the essential oil, thus changing the optical rotation. Nonetheless, compared to studies of commercial samples (Da Silva et al., 2013), these values were not very different in the two parameters.

Conclusions

The present study demonstrated that there is a wide variety of species and subspecies that produce *breu* oleoresin in the Erepecuru river area of the Amazon region, of the genus *Protium*, Burseraceae. In addition, a large variability in the chemical composition of the extracted essential oils was found. This study is a starting point for a future standardization of *breu* oleoresins as a raw material and also clarifies the differences between what is defined as white and black *breu* (trees and oleoresins), if there are any. We concluded that it is difficult to establish a relationship between "white *breu*" and "black *breu*" based on chemical, botanical or regional names. Several results should be highlighted: first, *P. heptaphyllum* March, which is characterized as a white *breu* tree in the literature, was characterized by the *quilombola* as a black *breu* tree and presented a different chemical composition; second, two different specimens of *P. decandrum* March collected at different sites were characterized by the *quilombola*, both as black (BBPIR) and white (WBB_1) *breu* trees; third, there is a large discrepancy in the chemical composition of the same oleoresin samples designated by the *quilombola* as black *breu*; and finally, there are organoleptic similarities between oleoresin samples belonging to the two different types of *breu*. Thus, the results indicate that the black or white designation is more cultural and regional than scientific. Apparently, for the *quilombola*, this difference is linked to the production volume, color aspect and scent of the oleoresin, while for scientists, this difference has not yet been determined and substantiated. Oleoresin aging by exposure to the environment, with subsequent volatilization of some components and oxidation of others, can be related to oleoresin organoleptic changes. Thus, with time, they become dimmer and friable, although they are white and tender at the time of leakage. This may also influence the *breu* oleoresin differentiation by the local community and researchers.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

All authors except for MFFM and HRB collected the plant materials and breu samples, MFFM identified plant specimens and contributed to the writing of the manuscript. The results presented here are part of the PhD thesis of ERS. ERS, DRO and HRB contributed to the extraction and analysis of the essential oils. SGL conceived the manuscript in its present form. ERS, DRO and SGL contributed

equally to the writing of this manuscript. All authors participated in the discussion of the ideas/conclusions presented here.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2016.05.003.

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