Composition of the essential oils from leaves of *Piper lepturum* Kunth (C.DC.) var. lepturum and *Piper lepturum* var. angustifolium (Miq.) Yunck. from Brazil

[Composición de los aceites esenciales de hojas de *Piper lepturum* Kunth (C.DC.) var. lepturum y *Piper lepturum* var. angustifolium (Miq.) Yunck. de Brasil]

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Abstract: The essential oils of Brazilian *Piper lepturum* var. lepturum and *Piper lepturum* var. angustifolium (Piperaceae) were obtained by hydrodistillation and analyzed by flame-detector gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS). According to GC and GC/MS analysis, the essential oils are mostly composed by sesquiterpenes hydrocarbons. β-Guaiene (29.96%) was the principal component in the essential oil of *P. lepturum* var. lepturum and β-Bisabolene (17.72%) was the principal components in the essential oil of *P. lepturum* var. angustifolium.

Keywords: β-Guaieno, β-Bisaboleno, Monoterpenos, Piperaceae, Sesquiterpenos

Resumen: Los aceites esenciales de las especies brasileñas *Piper lepturum* var. lepturum y *Piper lepturum* var. angustifolium fueron obtenidos por hidrodestilación y analizados utilizando cromatografía gas líquido con detector de ionización de llama (CG) y cromatografía gas líquido acoplada a un detector de masas (CG/EM). De acuerdo con los análisis de CG y CG/EM, los aceites esenciales muestran como componente principal β-Guaieno (29.96%) en el aceite esencial de *P. lepturum* var. lepturum y β-Bisaboleno (17.71%) en el aceite esencial de *P. lepturum* var. angustifolium.

Palabras clave: β-Guaieno, β-Bisaboleno, Monoterpenos, Piperaceae, Sesquiterpenos.

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INTRODUCTION
According to ethnobotanical surveys, in Brazil Piper species are widely employed in popular medicine; for example, P. aduncum Vell. is used to “cure” chronic ulcers, as astringent and diuretic; P. peltatum L. as diuretic and burns healing and P. marginatum Jacq. for liver inflammation. (Fonseca, 1940), two other species used in ethnomedicine are P. nigrum L. known as “pimenta do reino” employed as aromatic (Mors & Rizzini, 1966) and P. marginatum Jacq. that used for analgesic and anti-inflammatory properties (D’angelo et al., 1997). Indian tribe “waimiri-atroari” use macerated leaves of P. consanguineum Kunth. for wounds and snake bites, and there are other species of piper which are used for treating wounds caused by arrows (Milliken et al., 1986).

Recent studies indicate the importance of Piperaceae in ethnomedicine, including species that are found in Central and South America. For example, P. umbellatum L. is employed to treat different illnesses such as skin diseases, rheumatism, malaria and inflammations (Roersch, 2010; Silva et al., 2014a), P. amalago L. as diuretic and “kidney stone” (Novaes et al., 2014). For Indian tribe “Yanesha” in Peru, other Piper species are used to treat anxiety (Picard et al., 2014).

Chemical studies on Piper species have identified a large number of compounds (Parmar et al., 1997; Martins et al., 1998; Mesquita et al., 2005) with different biological activities such as cytotoxic, mutagenic, larvicidal, amoebicidal and antiviral (Péres et al., 2009; Matasih et al., 2011; Sauter et al., 2012; Pereira et al., 2013). In the chemical context, studies demonstrate the importance of Piper species to the knowledge of biologically active compounds, which can be found in essential oils and extracts (Silva et al., 2014b; Oliveira et al., 2014; Bagheri et al., 2014; Dal Picolo et al., 2014; Chithra et al., 2014).

Despite studies regarding the composition of essential oils of Piper species (Moura do Carmo et al., 2012; Do Nascimento et al., 2012; Oliveira et al., 2013), there is still a great amount of species, particularly from the Tropical Rain Forest, from which there are no chemical information.

This study aims to examine the composition of essential oils of Piper lepturum var. lepturum and P. lepturum var. angustifolium leaves in order to contribute to the phytochemical knowledge of Brazilian Piper species.

MATERIAL AND METHODS
Botanical material
Piper lepturum var. lepturum and P. lepturum var. angustifolium (Piperaceae) were collected in Tijuca Forest (S 22°58’01” W 43°14’48”), Rio de Janeiro, Brazil. The plants were identified by Elsí Franklin Guimarães and herbarium samples were deposited in the Botanical Garden Herbarium of Rio de Janeiro with registrations numbers RB 501326 e RB 501328, respectively.

Analysis of essential oils extracted from the plant material
Fresh leaves (100 g) cut into to small pieces were submitted to hydrodistillation in a modified Clevenger apparatus for two hours. Essential oil was extracted from the aqueous phase with 2 mL of dichloromethane, the resulting solutions was filtered over anhydrous sodium sulfate and transferred to amber amber flasks and kept at low temperature until analysis. Essential oils were analyzed by flame-detector gas chromatography (GC) coupled to mass spectrometry (GC/MS).

Essential oil analysis
Gas Chromatography (GC) analysis was performed using Varian Star 3400 CX equipped with fused silica capillary column DB-5 (30 m x 0.20 mm) and flame ionization detector, employing hydrogen as the carrier gas. The temperature program was from 60 to 240° C (3° C/min). The retention time (RT) was measured in minutes and the relative values of each compound in the mixture were obtained directly from the GC data.

Analysis by GC/MS was performed using Shimadzu QP2010 Plus at 70 eV provided with a ZB-5 MS column (30 m x 0.25 mm x 0.25 micrometers). The injector temperature was maintained at 260° C, interface at 200° C, and the operating temperature from 60 to 240° C (3° C/min). Helium was the carrier gas at 1 mL/min. The analyses were carried out at Center for Natural Products Research (NPPN), Federal University of Rio de Janeiro (UFRJ).

Analysis of the retention indexes and identification of compounds
Essential oil constituents were identified by calculating the retention indexes (RI) of each component, comparison of the mass spectra with
database (National Institute for Standard Technology - NIST-62,235 compounds) and literature (Adams, 2001). RIs were obtained based on the standard curve, obtained with elution times of components of a mixture composed of homologous series of n-alkanes with 6 to 26 carbon atoms.

RESULTS

The essential oil of *P. lepturum* var. *lepturum*, was characterized by the presence of sesquiterpenes, that gave account for 94.17% of the total oil. This oil has non-oxygenated and cyclic sesquiterpenes in its composition. The major components were β-guaiene (29.96%), germacrene B (23.76%), α - guaiene (10.91%), β - elemene (5.55%) and γ – elemene (4.21%). With regard to the chemical profile of *P. lepturum* var. angustifolium, minor monoterpene components were identified accounting for only 2.10% of the total oil and sesquiterpene components for 94.98%. The major components were: β - Bisabolene (17.72%), β - Caryophyllene (9.67%), Germacrene D (9.43%), α - Selinene (6.90%) and Germacrene B (6.85%) (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>RIC</th>
<th>RIL</th>
<th>Plvl (%)</th>
<th>Plva (%)</th>
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<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α – Tujene</td>
<td>929</td>
<td>931</td>
<td>-</td>
<td>0.12</td>
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<tr>
<td>α – Pinene</td>
<td>937</td>
<td>939</td>
<td>-</td>
<td>0.23</td>
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<tr>
<td>Sabinene</td>
<td>976</td>
<td>976</td>
<td>-</td>
<td>1.04</td>
</tr>
<tr>
<td>β – Pinene</td>
<td>981</td>
<td>980</td>
<td>-</td>
<td>0.29</td>
</tr>
<tr>
<td>Myrcene</td>
<td>990</td>
<td>991</td>
<td>-</td>
<td>0.42</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n.d.</td>
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<td></td>
<td>-</td>
<td>0.18</td>
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<tr>
<td>δ – Elemene</td>
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<td>1339</td>
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<td>0.48</td>
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<tr>
<td>α – Copaeene</td>
<td>1346</td>
<td>1351</td>
<td>-</td>
<td>0.42</td>
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<tr>
<td>β – Bourbonene</td>
<td>1381</td>
<td>1384</td>
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<tr>
<td>β – Elemene</td>
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<td>1391</td>
<td>5.55</td>
<td>3.34</td>
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<tr>
<td>α – Gurjunene</td>
<td>1403</td>
<td>1409</td>
<td>-</td>
<td>3.45</td>
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<tr>
<td>β – Cedrene</td>
<td>1409</td>
<td>1418</td>
<td>-</td>
<td>1.16</td>
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<tr>
<td>β – Caryophyllene</td>
<td>1418</td>
<td>1418</td>
<td>3.24</td>
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<tr>
<td>γ – Elemene</td>
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<td>1433</td>
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<td>1439</td>
<td>10.91</td>
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<tr>
<td>γ – Patchouline</td>
<td>1440</td>
<td>1441</td>
<td>-</td>
<td>0.96</td>
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<tr>
<td>α – Humulene</td>
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<td>3.27</td>
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<td>n.d.</td>
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<td>γ – Murolene</td>
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<td>Germacrene D</td>
<td>1482</td>
<td>1480</td>
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<td>9.43</td>
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<td>β - Selinene</td>
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<td>α – Selinene</td>
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<td>Bicyclogermacrene</td>
<td>1496</td>
<td>1494</td>
<td>-</td>
<td>6.62</td>
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<tr>
<td>β - Guaiene</td>
<td>1502</td>
<td>1500</td>
<td>29.96</td>
<td>-</td>
</tr>
<tr>
<td>α – Burnesene</td>
<td>1500</td>
<td>1505</td>
<td>-</td>
<td>6.45</td>
</tr>
<tr>
<td>n.d.</td>
<td>1505</td>
<td></td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>β – Bisabolene</td>
<td>1511</td>
<td>1509</td>
<td>-</td>
<td>17.72</td>
</tr>
</tbody>
</table>

Table 1

Compositions of essential oils from leaves of *P. lepturum* var. *lepturum* (Plvl) and *P. lepturum* var. angustifolium (Plva).
Pereira et al. Essential oils of Piper lepturum and P. lepturum var. angustifolium

α–Selinene 1517 1517 1.57 3.50
Cadina-1,4-diene 1535 1532 0.26 -
n.d. 1540 - 0.42 -
Germacrene-B 1559 1556 23.76 6.85
Ledol 1571 1565 0.29 -
Spathulenol 1577 1576 0.30 0.94
Caryophyllene oxide n.d. 1581 - 0.53
Germacrene B 1584 - 0.11 -
Globulol 1585 1583 - 0.34
Viridiflorol 1593 1590 3.32 0.50
Eudesmol <10-epi-γ> 1619 1619 0.25 -
Cedr-8(15)-em-9-alpha-ol 1644 1644 - 0.41

n.d. = not determined, \( \text{RI}_c \) = calculated retention index, \( \text{RI}_L \) = literature retention index

DISCUSSION
According to Andrade et al. (2011), representatives of Piperaceae usually have monoterpenes and sesquiterpenes as major constituents; however, Piper species of the Amazonia have a different composition with the presence of terpenoids and phenylpropanoids.

In different populations of P. aduncum, it was observed that there may be differences in the chemical profile of this species, and these results enabled the separation into two groups according to the major constituents, which may be classified as chemotypes (Potzernheim et al., 2012). Despite the intraspecific variations, essential oils can be used as phytochemical markers, for example in the identification of P. betle L. in India (Rawat et al., 1989) and also P. dilatatum from the Amazonia (Andrade et al., 2011).

In Piper species from São Tomé and Príncipe, the analysis of the essential oils of P. capense, P. nigrum and P. umbellatum showed chemical profile with predominance of monoterpenes, but for P. guineense, phenylpropanoids were the principal components (Martins et al., 1998). In another study, the chemical compositions of P. nigrum and P. guineense could be verified using other techniques such as solid-phase microextraction and based on these results, there was predominance of monoterpenes and sesquiterpenes (Jirovetz et al., 2002).

In fact, even in different locations, the presence of monoterpenes and sesquiterpenes can be considered an important marker for the genus Piper, considering that the analysis of the essential oil of both taxa revealed predominance of sesquiterpenes in leaves, which is characteristic of representatives of Piperaceae in the Atlantic Forest (Santos et al., 2001; Sperotto et al., 2013) and even in some species in the Amazonia rainforest (Morais et al., 2007; Silva et al., 2014a; Santos et al., 2014).

CONCLUSIONS
The composition of the essential oils of both species were determined for the first time and the principal components were sesquiterpenoids. The results of this work showed that β-guaiene is the major constituent of the essential oil of P. lepturum var. lepturum and β–bisabolene for P. lepturum var. angustifolium.

REFERENCES
Dal Picolo CR, Bezerra MP, Gomes KS, Passero...


