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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]

Flaviane Gomes Pereira[1], Paulo Roberto Dias dos Santos[2], Elsie Franklin Guimarães[2] & Maria Auxiliadora Coelho Kaplan[3]

[1]***Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Maracanã, Rio de Janeiro, RJ, Brasil21***
[2]***Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro, RJ, Brasil***
[3]***Universidade Federal do Rio de Janeiro, Núcleo de Pesquisa de Produtos Naturais, Cidade Universitária***
Contactos | Contacts: **Flaviane Gomes PEREIRA** - ***E-mail address:*** **flaviane.gp@gmail.com**

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; Matasioh *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 Elsie 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 1mL/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 1
Compositions of essential oils from leaves of *P. lepturum* var. lepturum (Plvl) and *P. lepturum* var. angustifolium (Plva).

Compound	RI_C	RI_L	Plvl (%)	Plva (%)
Monoterpenes				
α – Tujene	929	931	-	0.12
α – Pinene	937	939	-	0.23
Sabinene	976	976	-	1.04
β – Pinene	981	980	-	0.29
Myrcene	990	991	-	0.42
Sesquiterpenes				
n.d.	1330	-	-	0.18
δ – Elemene	1335	1339	0.11	0.48
α – Cubebene	1346	1351	-	0.42
α – Copaene	1374	1376	0.48	1.56
β – Bourbonene	1381	1384	0.24	0.49
β – Elemene	1389	1391	5.55	3.34
α – Gurjunene	1403	1409	-	3.45
β – Cedrene	1409	1418	-	1.16
β – Caryophyllene	1418	1418	3.24	9.67
γ – Elemene	1436	1433	4.21	1.59
α – Guaiene	1437	1439	10.91	3.61
γ – Patchoulene	1440	1441	-	0.96
α – Humulene	1454	1454	3.27	4.56
n.d.	1459	1455	0.26	-
γ – Muurolene	1477	1477	2.67	-
Germacrene D	1482	1480	-	9.43
β-Selinene	1486	1485	2.79	1.15
α – Selinene	1487	1494	1.24	6.90
Bicyclogermacrene	1496	1494	-	6.62
β-Guaiene	1502	1500	29.96	-
α – Burnesene	1500	1505	-	6.45
n.d.	1505	-	1.00	-
β – Bisabolene	1511	1509	-	17.72

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	1580	1581	-	0.53
n.d	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, RI_{C} = calculated retention index, 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*, phenyl-propanoids 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.

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