ACTA
AMAZONICA | http://dx.doi.org/10.1590/1809-4392201800771

ORIGINAL ARTICLE

First record of the chemical composition of essential oil of *Piper bellidifolium*, *Piper durilignum*, *Piper acutilimbum* and *Piper consanguineum* from the Brazilian Amazon forest

Carolina Alves de ARAUJO[1], Claudio Augusto Gomes da CAMARA[1]*, Marcilio Martins de MORAES[1], Geraldo José Nascimento de VASCONCELOS[2], Marta Regina Silva PEREIRA[3], Charles Eugene ZARTMAN[3]

[1] Universidade Federal Rural de Pernambuco, Departamento de Química. Rua Dom Manoel de Medeiros s/n, CEP 52171-030 Recife, PE, Brasil

[2] Universidade Federal do Amazonas, Campus ICET. Rua Nossa Senhora do Rosário, 3863, Tiradentes, CEP 69103-128, Itacoatiara, Amazonas, Brasil

[3] Instituto Nacional de Pesquisa da Amazônia, Coordenação de Biodiversidade. Av. André Araújo, 2936, Petrópolis, CEP 69060-001, Manaus, Amazonas, Brasil

* Corresponding author: claudio_agc@hotmail.com

ABSTRACT

Piper bellidifolium, *Piper durilignum*, *Piper acutilimbum* and *Piper consanguineum* are bushes that occur in the Amazon and are morphologically similar. With the aim of analyzing the chemical profile of the volatile constituents of these species, essential oils from the leaves were obtained through steam distillation and analyzed using gas chromatography–flame ionization detection (GC-FID) and gas chromatograph coupled to a mass spectrometer (GC-MS). The chemical analysis enabled the identification of 95 compounds representing 96.3 ± 0.6% of the *P. bellidifolium* oil, 95.5 ± 0.71% of the *P. durilignum* oil, 98.0 ± 1.0% of the *P. acutilimbum* oil and 96.1 ± 2.1% of the *P. consanguineum* oil. Although sesquiterpenes were the predominant chemical class in the oils of the four species, qualitative and quantitative differences were found in their chemical composition. The major constituents were (*E*)-nerolidol (20.3 ± 0.4%) in the *P. bellidifolium* oil, germacrene D (11.1 ± 0.3%) in the *P. durilignum* oil, and γ-eudesmol in both the *P. consanguineum* (18.6 ± 0.5%) and *P. acutilimbum* (7.5 ± 0.4%) oils. Despite their morphological similarity, a principal component analysis (PCA) of the GC-MS data clearly separated the four species according to the chemical profile of the essential oil extracted from their leaves.

KEYWORDS: Amazon biome, *Piper* ssp, (*E*)-Nerolidol, Germacrene D, γ-Eudesmol

Primeiro registro da composição química de óleos essenciais de *Piper bellidifolium*, *Piper durilignum*, *Piper acutilimbum* e *Piper consanguineum* da Floresta Amazônica no Brasil

RESUMO

Piper bellidifolium, *Piper durilignum*, *Piper acutilimbum* e *Piper consanguineum* são arbustos que ocorrem na Amazônia e são morfologicamente similares. Com o intuito de analisar o perfil químico dos constituintes voláteis dessas espécies, óleos essenciais das folhas foram obtidos por hidrodestilação e analisados por cromatografia gasosa - detector por ionização de chama (CG-FID) e cromatografia gasosa acoplada a espectrometria de massa (CG-EM). A análise química permitiu identificar 95 compostos, representando 96.3 ± 0.6% do óleo de *P. bellidifolium*; 95.5 ± 0.71% de *P. durilignum*; 98.0 ± 1.0% de *P. acutilimbum* e 96.1 ± 2.1% de *P. consanguineum*. Apesar dos óleos das quatro espécies terem sesquiterpeno como classe química predominante, diferenças qualitativas e quantitativas em sua composição química foram observadas. Os principais componentes encontrados foram: (*E*)-nerolidol (20.3 ± 0.4%) em *P. bellidifolium*; germacreno D (11.1 ± 0.3%) em *P. durilignum*; e γ-eudesmol nos óleos de *P. consanguineum* (18.6 ± 0.5%) e *P. acutilimbum* (7.5 ± 0.4%). Apesar da similaridade morfológica entre as espécies, uma análise de componentes principais (PCA) dos dados de CG-EM claramente separou as quatro espécies quanto ao perfil químico do óleo essencial extríado de suas folhas.

PALAVRAS-CHAVE: bioma Amazônia, *Piper ss*p, (*E*)-Nerolidol, Germacreno D, γ-Eudesmol

CITE AS: Araujo, C.A. de; Camara, C.A.G. da; Moraes, M.M. de; Vasconcelos, G.J.N. de; Pereira, M.R.S.; Zartman, C.E. 2018. First record of the chemical composition of essential oil of *Piper bellidifolium*, *Piper durilignum*, *Piper acutilimbum* and *Piper consanguineum* from in the Brazilian Amazon forest. *Acta Amazonica* 48: 330-337.

INTRODUCTION

Piperaceae is considered one of the most basal clades among angiosperms encountered in tropical and subtropical regions (Frodin 2004). Among the genera belonging to the family, *Piper* is by far the largest, with nearly 2000 species found in both hemispheres in tropical and temperate regions (Machado 2007; Quijano-Abril *et al.* 2006). In Brazil, approximately 290 species occur throughout the country, with a greatest representation in the equatorial north, as demonstrated by the 136 species with registered occurrences in the state of Amazonas (Guimarães *et al.* 2017). However, different authors report an excessive and unproven multiplication of species names for *Piper*, which hinders an objective evaluation of the true number of species of the genus (Ruschel 2004).

The leaves of many species of *Piper* are used in folk medicine in the form of infusions for the treatment of ailments and also have economic importance due to their culinary uses and the production of essential oils (Gogosz *et al.* 2012). Phytochemical investigations of different *Piper* species and plant parts have led to the isolation of numerous active components, such as alkaloids, flavonoids, lignans and essential oils (Santana *et al.* 2015; Morais *et al.* 2007). These oils are basically composed of phenylpropanoids, such as safrole, dillapiole and myristicin, and/or terpenes, such as limonene, β-caryophyllene, spathulenol, (*E*)-nerolidol, bicyclogermacrene and α-cadinol (Guerrini *et al.* 2009; Santos *et al.* 2001; Maia and Andrade 2009). Investigations of the biological properties of essential oils from this genus have revealed antimicrobial (Oliveira *et al.* 2016), antioxidant (Woguem *et al.* 2013), acaricidal (Araújo *et al.* 2012) and insecticidal activities (Santana *et al.* 2015).

According to Guimarães *et al.* (2017), approximately 80% of the species of *Piper* registered for the Brazilian state of Amazonas have not been submitted to studies on the chemical composition of their essential oils. Such is the case of *P. acutilimbum* C. DC.*, P. consanguineum* (Kunth) Trel. & Yunck.*, P. durilignum* C. DC. and *P. bellidifolium* Yunck., which occur in the municipalities of Rio Preto da Eva and Manaus in the state of Amazonas and are popularly known as long pepper (pimenta longa) and monkey pepper (pimenta de macaco) due to the length of their inflorescences. These plants are bushes that are morphologically quite similar. Despite this morphological similarity, there are no synonyms described for these species.

As part of a survey of the aromatic flora of Amazonia, this work offers the first description of the chemical composition of essential oils from leaves of the species *P. acutilimbum, P. consanguineum, P. durilignum* and *P. bellidifolium.*

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *Piper acutilimbum* C.DC. and *Piper durilignum* C.DC. were collected in Rio Preto da Eva, metropolitan region of Manaus (02°44'00"S, 59°47'26"W). *Piper bellidifolium* Yunk. was collected in Itacoatiara, metropolitan region of Manaus (03°01'42"S, 58°42'37"W). *Piper consanguineum* (Kunth) Trel. & Yunck. was collected in the Adolpho Ducke Reserve in Manaus, Amazonas (02°57'18"S, 59°55'41"W). Samples were taken from three individual plants of each species. The plants were identified by botanist Marta Regina Silva Pereira, of Instituto Nacional de Pesquisas da Amazônia (INPA). Vouchers of samples were mounted and deposited in the INPA herbarium, under numbers 673 (*Piper acutilimbum*), 680 (*Piper consanguineum*), 674 (*Piper durilignum*) and 694 (*Piper bellidifolium*).

Chemicals

All monoterpenes (β-Pinene, Limonene, 1,8-Cineole, Terpinolene, Terpinen-4-ol e α-Terpineol), and sesquiterpenes (α-Copaene, β-Caryophyllene, Aromadendrene, α-Humulene, Germacrene D, (*E*)-Nerolidol and Spathulenol) were purchased from Sigma-Aldrich – Brazil and used for co-injetion to confirm the chemical identification.

Essential oil extraction and GC-FID analysis

The essential oils from fresh leaves (100 g from each of three plants of each species), were separately isolated using a modified Clevenger-type apparatus and hydrodistillation for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept at low temperature (-5 ºC) until analysis. Total oil yields were expressed as percentages (grams of oil per grams of fresh plant material). Quantitative GC (500 GC, PerkinElmer Clarus, Shelton, CO, USA) analyses were carried out using an apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness) (J & W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate 3 °C min^{-1}. Injector and detector temperature was 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} in split mode (1:30). The injection volume was 0.5 µL of diluted solution (1/100) of oil in n-hexane. The amount of each compound was calculated from GC-FID peak areas in the order of DB-5 column elution and expressed as a relative percentage of the total area of the chromatograms. All analyses were carried out in triplicate.

GC-MS analysis

The qualitative gas chromatography-mass spectrometry (GC-MS) (220-MS IT GC, Varian, Walnut Creek, CA, USA) analyses were carried out using a system with a mass selective detector, mass spectrometer in EI 70 eV with a scan interval of 0.5 s and fragments from 40 to 550 Da fitted with the same column and temperature program as that for the GC-FID analyses, with the following parameters: carrier gas = helium; flow rate = 1 mL min^{-1}; split mode (1:30); injected volume = 1 µL of diluted solution (1/100) of oil in n-hexane.

Identification of components

Identification of the components was based on GC-MS retention indices with reference to a homologous series of C8-C40 n-alkanes calculated using the Van der Dool and Kratz equation (Van den Dool and Kratz 1963) and by computer matching against the mass spectral library of the GC-MS data system (NIST 14 and WILEY 11th) and co-injection with authentic standards as well as other published mass spectra (Adams 2007). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Principal component analysis

Principal component analysis (PCA) based on the complete data set (all parameters measured and the three independent samples for each species) was conducted to evaluate the chemical variation of essential oils within and among the four species. The GC-MS data were exported in ASCII format to Microsoft Excel to produce a data matrix of sample versus metabolite peak with associated peak areas. All the analyses were performed using the Unscrambler® software version 9.5 (CAMO Process AS, Norway, 1996-2007).

RESULTS

The steam distillation of the leaves yielded yellowish oils with critic aromas. The greatest yield was achieved with *P. consanguineum* (0.30 ± 0.02%), followed by *P. acutilimbum* (0.18 ± 0.01%), *P. durilignum* (0.12 ± 0.00%) and *P. bellidifolium* (0.01 ± 0.00%) (Table 1).

The GC-MS analysis enabled the identification of 95 compounds representing 96.3 ± 0.6% of the chemical composition of the oil from *P. bellidifolium,* 95.5 ± 0.71% of *P. durilignum,* 98.0 ± 1.0% of *P. acutilimbum* and 96.1 ± 2.1% of *P. consanguineum*. All *Piper* oils were composed of monoterpenes and sesquiterpernes. *Piper bellidifolium* (95.1 ± 0.5%), *P. durilignum* (72.2 ± 0.3%), *P. acutilimbum* (97.3 ± 1.1%) and *P. consanguineum* (95.5 ± 0.4%) oils were mainly composed of sesquiterpenes (Table 1).

Forty-one constituents were identified for the *P. bellidifolium* oil, in which (*E*)-nerolidol (20.3 ± 0.4%) was the major constituent, followed by aromadendrene (13.3 ± 0.3%) and α-copaene (10.9 ± 0.2%). Hinesol (5.7 ± 0.1%), longifolene (5.4 ± 0.1%) and β-acoradiene (5.0 ± 0.1%) were also found in significant quantities in this oil (Table 1). With 47 constituents identified, representing 98.0 ± 1.0% of the total, the *P. acutilimbum* oil had the largest percentage of sesquiterpenes (97.3 ± 1.1%). The major constituents were γ-eudesmol (7.5 ± 0.4%), germacrene B (6.9 ± 1.7%), α-muurolol (6.4 ± 0.1%), β-longipinene (6.2 ± 0.1%) and 1-*epi*-cubenol (5.6 ± 0.2%). Forty compounds were identified, representing 96.1 ± 2.1% of the *P. consanguineum* oil. The most abundant compound was γ-eudesmol (18.6 ± 0.5%), followed by γ-cadinene (11.3 ± 0.1%), (*E*)-nerolidol (6.2 ± 0.0%) and α-muurolol (5.0 ± 0.2%). Thirty-eight compounds were identified in the *P. durilignum* oil, the major constituents of which were germacrene D (11.1 ± 0.3%), limonene (10.7 ± 0.5%) and β-caryophyllene (9.1 ± 0.2%). This oil had the highest percentage of monoterpenes among the oils analyzed (23.3 ± 0.5%). Other constituents were also identified in quantities higher than 5%: (*E*)-nerolidol (6.2 ± 0.2%), *epi*-α-cadinol (5.2 ± 0.1%) and linalool (5.1 ± 0.1%).

γ-Eudesmol was found in a greater proportion in the *P. consanguineum* oil (18.6 ± 0.5%), followed by *P. acutilimbum* (7.5 ± 0.4%), *P. bellidifolium* (2.3 ± 0.0%) and *P. durilignum* (0.9 ± 0.0%). Germacrene D was found in a greater proportion in the *P. durilignum* oil (11.1 ± 0.3%) and at less than 4% in the other species. 9-*Epi*-(*E*)-caryophyllene and *allo*-aromadendrene epoxide were found at less than 3% in all species.

The PCA grouped the samples closely within species and separated the samples clearly among the four species (Figure 1). Seventy-two percent of the variability in the data was explained by the first (PC1 = 47%) and second (PC2 = 25%) components.

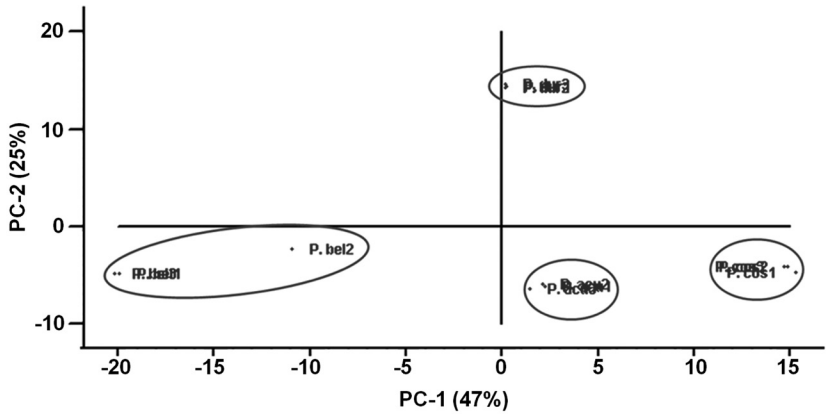

Figure 1. Principal component analysis scores (PC1 and PC2) of the GC-MS of essential oil of leaves of *P. bellidifolium* (P.bel), *P. durilignum* (P.dur), *P. acutilimbum* (P.acu) and *P. consanguineum* (P.cos).

Table 1. Percentage composition yield of essential oils from leaves of *Piper bellidifolium* (P.bel), *Piper durilignum* (P.dur), *Piper acutilimbum* (P.acu) and *Piper consanguineum* (P.con).

Compound	RI[a]	RI[b]	P.bel	P.dur	P.acu	P.con
Yield (%) ± SD			0.01 ± 0.00	0.12 ± 0.00	0.18 ± 0.01	0.30 ± 0.02
α-Thujene	929	924	--	2.0 ± 0.1	--	--
β-Pinene*	974	974	--	5.0 ± 0.1	--	--
δ-3-Carene	1006	1008	0.3 ± 0.0	--	--	--
Limonene*	1022	1024	0.5 ± 0.0	10.7 ± 0.5	--	--
Terpinolene	1085	1086	--	--	0.2 ± 0.0	0.6 ± 0.0
Linalool	1095	1095	0.3 ± 0.0	5.1 ± 0.1	--	--
α-Terpineol*	1180	1186	0.2 ± 0.0	0.4 ± 0.0	--	--
y-Terpineol	1191	1199	--	--	0.6 ± 0.0	--
Neo-3-Thujanol acetate	1270	1273	--	0.1 ± 0.0	--	--
α-Cubebene	1339	1345	0.3 ± 0.0	--	0.1 ± 0.0	--
Cyclosativene	1358	1369	0.2 ± 0.0	--	--	--
α-Ylangene	1370	1373	--	4.1 ± 0.1	0.7 ± 0.0	--
Isoledene	1374	1374	--	--	0.4 ± 0.0	1.1 ± 0.0
α-Copaene*	1376	1374	10.9 ± 0.2	--	--	--
β-Panasinsene	1381	1381	--	5.1 ± 0.1	0.4 ± 0.0	1.3 ± 0.0
β-Cubebene	1386	1387	--	--	0.6 ± 0.0	--
β-Elemene	1390	1389	0.4 ± 0.0	--	--	--
β-Longipinene	1399	1400	--	--	6.2 ± 0.1	--
Longifoliene	1405	1407	5.4 ± 0.1	--	--	--
β-Funebrene	1413	1413	--	--	0.2 ± 0.0	1.0 ± 0.0
β-Caryophyllene*	1417	1417	--	9.1 ± 0.2	--	0.5 ± 0.0
β-Cedrene	1421	1419	0.7 ± 0.1	0.7 ± 0.0	0.4 ± 0.1	--
β-Copaene	1426	1430	--	0.7 ± 0.0	--	--
α-*Trans*-bergamotene	1430	1432	--	--	2.7 ± 0.0	--
y-Elemene	1435	1434	--	2.9 ± 0.0	--	--
α-Guaiene	1438	1437	--	--	0.3 ± 0.0	2.0 ± 0.0
Aromadendrene*	1440	1439	13.3 ± 0.3	1.6 ± 0.1	--	0.6 ± 0.0
Cis-Muurola 3,5-diene	1444	1448	0.8 ± 0.0	--	--	3.8 ± 0.1
α-Humulene*	1447	1452	--	--	0.3 ± 0.0	--
α-Patchoulene	1449	1454	--	--	0.7 ± 0.0	1.3 ± 0.0
allo-Aromadendrene	1460	1458	1.1 ± 0.1	--	--	1.1 ± 0.0
Dehydro-Aromadendrane	1462	1460	0.5 ± 0.0	--	--	--
9-*epi*-(*E*)-Caryophyllene	1464	1464	0.3 ± 0.0	1.2 ± 0.0	3.0 ± 0.0	1.0 ± 0.0
β-Acoradiene	1476	1469	5.0 ± 0.1	--	--	--
y-Muurolene	1480	1478	--	--	--	1.4 ± 0.0
y-Himachalene	1482	1481	3.7 ± 0.0	--	2.6 ± 0.0	1.1 ± 0.0
Germacrene D*	1484	1484	1.7 ± 0.1	11.1 ± 0.3	0.5 ± 0.0	3.2 ± 0.1
β-Selinene	1485	1489	3.6 ± 0.1	3.3 ± 0.1	--	--
Epi-Cubebol	1498	1493	0.4 ± 0.0	--	3.8 ± 0.0	0.9 ± 0.0
Trans-*β*-Guaiene	1504	1502	1.1 ± 0.0	1.6 ± 0.1	--	--
α-Bulnesene	1506	1509	--	--	0.6 ± 0.0	--
y-Cadinene	1515	1513	--	--	0.6 ± 0.0	11.3 ± 0.1
(*Z*)-*y*-Bisabolene	1515	1514	--	--	--	0.8 ± 0.1
Cubebol	1516	1514	--	--	1.0 ± 0.0	--
α-Dehydro ar-Himachalene	1518	1516	--	--	1.4 ± 0.6	--
7-*epi*-*α*-Selinene	1520	1520	--	--	1.0 ± 0.1	--
Trans-Calamenene	1523	1521	0.4 ± 0.1	--	1.2 ± 0.2	--
δ-Cadinene	1523	1522	0.6 ± 0.1	--	4.4 ± 1.5	--
y-Cuprenene	1534	1532	--	0.8 ± 0.0	--	--

Table 1. Continued.

Compound	RI[a]	RI[b]	P.bel	P.dur	P.acu	P.con
10-*epi*-Cubebol	1534	1533	0.4 ± 0.0	--	3.0 ± 0.0	1.5 ± 0.0
Selina-3-7(11)-diene	1540	1545	--	--	0.3 ± 2.4	--
Italiene epoxide	1545	1547	--	0.6 ± 0.1	3.0 ± 1.3	--
Elemol	1550	1548	--	2.4 ± 0.0	4.1 ± 2.4	--
Germacrene B	1554	1559	--	2.7 ± 0.0	6.9 ± 1.7	1.2 ± 0.1
(*E*)-Nerolidol*	1561	1561	20.3 ± 0.4	6.2 ± 0.2	--	6.2 ± 0.0
Maaliol	1565	1566	--	0.9 ± 0.0	1.6 ± 0.4	0.7 ± 0.1
Palustrol	1570	1567	--	--	--	3.4 ± 0.2
Longipinanol	1571	1567	1.9 ± 0.1	--	--	1.2 ± 0.2
α-Cedrene epoxide	1573	1574	3.6 ± 0.2	--	0.7 ± 0.0	--
Spathulenol*	1576	1577	--	--	1.4 ± 0.0	--
Trans-Sesquisabinene hidrate	1577	1577	--	--	1.6 ± 0.0	--
Himachalene epoxide	1580	1578	--	--	0.7 ± 0.1	--
Caryophyllene oxide	1586	1582	--	--	4.2 ± 0.1	--
Globulol	1595	1590	--	1.6 ± 0.1	1.5 ± 0.2	3.7 ± 0.1
Viridiflorol	1597	1592	--	0.4 ± 0.0	1.4 ± 0.3	1.0 ± 0.1
Carotol	1599	1594	--	--	4.6 ± 0.8	4.2 ± 0.1
Longiborneol	1600	1599	0.8 ± 0.0	--	2.9 ± 0.1	--
Cedrol	1600	1600	--	--	4.1 ± 0.1	1.1 ± 0.0
Guaiol	1603	1600	--	1.1 ± 0.1	--	1.8 ± 0.0
Ledol	1604	1602	--	0.6 ± 0.0	--	--
1,10-di-*epi*-Cubenol	1612	1618	1.1 ± 0.1	1.5 ± 0.1	--	1.2 ± 0.0
β-Cedrene epoxide	1625	1621	--	0.8 ± 0.0	--	--
1-*epi*-Cubenol	1631	1627	1.2 ± 0.0	0.8 ± 0.0	5.6 ± 0.2	--
γ-Eudesmol	1635	1630	2.3 ± 0.0	0.9 ± 0.0	7.5 ± 0.4	18.6 ± 0.5
epi-*α*-Cadinol	1638	1638	--	5.2 ± 0.1	--	--
allo-Aromadendrene epoxide	1640	1639	1.3 ± 0.1	0.9 ± 0.0	0.6 ± 0.0	1.2 ± 0.1
Hinesol	1640	1640	5.7 ± 0.1	--	--	--
α-Muurolol	1650	1644	--	1.0 ± 0.1	6.4 ± 0.1	5.0 ± 0.2
Cubenol	1652	1645	--	--	--	1.7 ± 0.0
Pogostol	1656	1651	--	--	--	1.5 ± 0.4
Valerianol	1660	1656	--	--	--	1.2 ± 0.1
cis-Calamenen-10-ol	1665	1660	0.4 ± 0.0	0.6 ± 0.0	0.3 ± 0.1	--
Intermedeol	1668	1665	0.5 ± 0.0	--	--	--
14-hydroxy (*Z*)-Caryophyllene	1670	1666	--	0.7 ± 0.0	0.6 ± 0.3	4.6 ± 0.2
(*E*)-Bisabol-11-ol	1672	1667	0.7 ± 0.0	--	--	--
14-hydroxy-9-*epi*-(*E*)- Caryophyllene	1673	1668	0.7 ± 0.0	--	--	--
Khusinol	1683	1679	--	0.6 ± 0.0	--	--
Germacra-4(15), 5,10(14)-trien-1-α-ol	1689	1685	--	0.6 ± 0.0	--	--
(*Z*)-*α*-*trans*-Bergamotol	1686	1690	1.4 ± 0.1	--	--	--
Eudesm-7(11)-em-4-ol	1699	1700	0.7 ± 0.1	--	--	--
Amorpha-4,9-dien-2-ol	1701	1700	1.2 ± 0.1	--	--	--
Cis-Thusopsenal	1712	1708	0.2 ± 0.0	--	--	0.1 ± 0.0
14-hydroxy-α-Humulene	1719	1713	--	--	--	0.3 ± 0.0
Vetiselinenol	1734	1730	--	--	--	0.5 ± 0.1
Epi-Cyclocolorenone	1768	1774	--	--	--	0.9 ± 0.1
Monoterpenes			1.2 ± 0.0	23.3 ± 0.5	0.7 ± 0.0	0.6 ± 0.0
Sesquiterpenes			95.1 ± 0.5	72.2 ± 0.3	97.3 ± 1.1	95.5 ± 0.4
Total			96.3 ± 0.6	95.5 ± 0.7	98.0 ± 1.0	96.1 ± 0.4

RI[a] = Retention indices calculated from retention times in relation to those of a series C_8–C_{40} of n-alkanes on a 30m DB-5 capillary column; RI[b] = Retention indices from the literature. RI = retention index; MS = mass spectroscopy; CI: Co-injection with authentic compounds. Method of identification: RI, MS and *RI, MS, CI.

DISCUSSION

The yields obtained in this study are in agreement with those reported in the literature for leaf oils from other species of *Piper* collected in Amazonia. Morais *et al.* (2007) and Rameshkumar *et al*. (2011) report yields of 0.01% for *P. gaudichaudianum* and 0.05% for *P. longum*, which are similar to that found for *P. bellidifolium*. Andrade and Zoghbi (2007) report a yield of 0.03% for *P. glandulosissimum* (0.3%), which is similar to that found for *P. consanguineum*. The differences in yields among our four species are likely due to the influence of abiotic factors, such as temperature, luminosity, seasonality, nutrition and water availability (Pacheco *et al.* 2016). Further analyses using samples from a wider geographical scale are necessary to reliably determine the inter-specific variability in leaf oil yield in these species.

There were significant qualitative differences in the chemical profiles among the oils of the four species, as was evident in the PCA analysis. Among the 95 compounds identified, only four were common to all four oils (germacrene D, 9-*epi*-(*E*)-caryophyllene, γ-eudesmol and *allo*-aromadendrene epoxide). The main compounds identified in this study have also been found in other *Piper* species in different regions of Brazil and the world. For instance, germacrene D has been found in large quantities in leaf oil of *P. magnibaccum* (40.8%) from Malaysia (Hashim *et al.* 2017) and *P. pedicellatum* (40.80%) from India (Saika *et al.* 2015). In Brazil, this sesquiterpene has been identified as a major constituent of the oils from *P. regnellii* (45.6 to 51.4%), *P. umbellatum* (55.8%) and *P. arboreum* (72.87%), which occur in the states of São Paulo (Anderson *et al.* 2017; Perigo *et al.* 2016) and Rondônia (Machado *et al.* 1994).

(*E*)-nerolidol, which was the major constituent of the *P. bellidifolium* oil, is also reported to be the major constituent of the leaf oils from *P. claussenianum* (80%) from the state of Espírito Santo, Brazil (Marques *et al.* 2017), *P. gaudichaudianum* (22.06%) from the state of Rio Grande do Sul, Brazil (Sperotto *et al.* 2013) and *P. flaviflorum* (40.5%) from China (Li *et al.* 2014). γ-Eudesmol, which was the major constituent of the *P. acutilimbum* and *P. consanguineum* oils, has also been reported to be the main component of the leaf oil of *P. duckei* (17.9%) from the state of Amazonas, Brazil (Carmo *et al.* 2012), *P. arboretum* (14.61%) from the state of Rio de Janeiro, Brazil (Santos *et al.* 2001) and *P. cernuum* (11.65%) from the state of Santa Catarina, Brazil (Gasparetto *et al.* 2016).

Other constituents found in significant percentages in the *Piper* oils investigated herein have been characterized as major constituents in leaf oils from several species of the genus collected in all regions of Brazil. For example, β-caryophyllene, which was identified in *P. durilignum*, has also been found in the leaf oil of *P. cyrtopodon* (34.6%) occurring in northern Brazil (Andrade *et al.* 2006), *P. dilatatum* (25.03%) from northeastern Brazil (Cysne *et al.* 2005), and *P. gaudichaudianum* (17.4%) from southern Brazil (Von Poser *et al.* 1994), as well as in *P. arboreum* (25.1%) (Navickiene *et al.* 2006), *P. cernuum* (20.69%) (Costantin *et al.* 2001) and *P. truncatum* (24.2%) (Trindade *et al.* 2010) from southeastern Brazil. β-Caryophyllene has also been reported as a major constituent of *Piper* species in other countries, such as *P. longispicum* (45.2%) in Panamá (Santana *et al.* 2015), *P. hispidum* (23.6%) in Colombia (Benitez *et al.* 2009), *P. umbellatum* (28.2%) in Cameroon (François *et al.* 2009), *P. chaba* (28.6%) in India (Rameshkumar *et al.* 2011), and *P. nigrum* (24.34%) in Malaysia (Bagheri *et al.* 2014). Limonene, which was found in *P. durilignum*, has been reported as a major constituent of leaf oils from *P. vitaceum* (33.2%) in the state of Amazonas, Brazil (Luz *et al.* 2000) and *P. confertinodum* (18.3%) in Colombia (Caballero-Gallardo *et al.* 2014).

The sesquiterpene α-copaene, which was present at over 10% in the *P. bellidifolium* oil, has also been reported as a major constituent of *P. boehmeriafolium* oil (28.3%) from Vietnam (Hieu *et al.* 2014). Aromadendrene was another sesquiterpene present in significant proportion in *P. belldifolium* and has been reported as a major constituent of the leaf oils of *P. gaudichaudianum* (15.55%) from the state of Rondônia, Brazil (Morais *et al.* 2007) and *P. muricatum* (16.2%) from Malaysia (Salleh *et al.* 2015). The sesquiterpene γ-cadinene has been characterized in the leaf oil of *P. hispidum* (25.13%) from the state of Rondônia, Brazil (Machado *et al.* 1994) and *P. cubeba* (16.6%) from Indonesia (Bos *et al.* 2007) in larger quantities than that found by us in the oil of *P. consanguineum*.

CONCLUSIONS

This is the first report of the chemical composition of essential oils from *Piper bellidifolium*, *P. durilignum, P. acutilimbum* and *P. consanguineum* occurring in the Amazon region in Brazil. The abundance of sesquiterpenes in the leaf oils from these species is in agreement with the predominant class reported in the literature for species of *Piper*. The species were clearly differentiated by their qualitative chemical compositions, with only four constituents common to the four species.

ACKNOWLEDGMENTS

The authors are grateful to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for awarding a grant (CAPES proc. # IBPG-0984-5.01/10); a productivity scholarship (CNPq, # 312277/2013-0), and research funding for this study (CNPq proc. # 403162/203-0; FACEPE proc. # APQ-1008-1.06/15; APQ-0476-1.06/14; APQ-08601.06/16).

REFERENCES

Adams, R.P. 2007. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Espectroscopy*, 4th ed. Allured Publishing Corporation, Carol Stream, Illinois, 804p.

Andrade, E.H.A.; Guimarães, E.F.; da Silva, M.H.L.; Pereira, R.A.; Bastos, C.N.; Maia, J.G.S. 2006. Essential Oil Composition of *Piper cyrtopodon* (Miq.) C. DC. *Journal of Essential Oil Bearing Plants,* 9: 53-59.

Andrade, E.H.A.; Zoghbi, M.G.B. 2007. Volatile Constituents of the Leaves and Stems of *Piper glandulosissimum* Yunck. *Journal of Essential Oil Research,* 19: 401-402.

Anderson, R.R.; Girola, N.; Figueiredo, C.R.; Londero, V.S.; Lago, J.H.G. 2017. Circadian variation and *in vitro* cytotoxic activity evaluation of volatile compounds from leaves of *Piper regnellii* (Miq) C. DC. var. *regnellii* (C. DC.) Yunck (Piperaceae). *Natural Product Research,* 3: 1-4.

Araújo, M.J.C.; Da Camara, C.A.G.; Born, F.S.; Moraes, M.M.; Badji, C.A. 2012. Acaricidal activity and repellency of essential oil from *Piper aduncum* and its components against *Tetranychus urticae*. *Experimental and Applied Acarology,* 57: 139-155.

Bagheri, H.; Manap, M.Y.B.A.; Solati, Z. 2014. Response surface methodology applied to supercritical carbon dioxide extraction of *Piper nigrum* L. essential oil. *Food Science and Technology* (*LEB*), 57: 149-155.

Benítez, N.P.; Melendez, E.; Stashenko, E.E. 2009. Composición química y actividad antibacteriana del aceite esencial de hojas de *Piper lanceaefolium,* planta usada tradicionalmente en Colombia. *Boletin Latinoamericano y del Caribe de Plantas*, 8: 301-304.

Bos, R.; Woerdenbag, H.J.; Kayser, O.; Quax, W.J. 2007. Essential Oil Constituents of *Piper cubeba* L. fils. from Indonesia. *Journal of Essential Oil Research*, 19: 14-17.

Caballero-Gallardo, K.; Pino-Benitez, N.; Pajaro-Castro, N.; Stashenko, E.; Olivero-Verbel, J. 2014. Plants cultivated in Choco, Colombia, as source of repellents against *Tribolium castaneum* (Herbst). *Journal of Asia-Pacific Entomology,* 17: 753-759.

Costantin, M.B.; Sartorelli, P.; Limberger, R.; Henriques, A.T.; Steppe, M.; Ferreira, M.J.P.; Ohara, M.T.; Emerenciano, V.P.; Kato, M.J. 2001. Essential Oils from *Piper cernuum* and *Piper regnellii*: Antimicrobial Activities and Analysis by GC/MS and ^{13}C-NMR. *Planta Medica,* 67: 771-773.

Carmo, D.F.M.; Amaral, A.C.F.; Machado, G.M.C.; Leon, L.L.; Silva, J.R.A. 2012. Chemical and Biological Analyses of the Essential Oils and Main Constituents of *Piper* Species. *Molecules,* 17: 1819-1829.

Cysne, J.B.; Canuto, K.M.; Pessoa, O.D.L.; Nunes, E.P.; Silveira, E.R. 2005. Leaf Essential Oils of Four *Piper* Species from the State of Ceará - Northeast of Brazil. *Journal of the Brazilian Chemical Society,* 16: 1378-1381.

Francois, T.; Michel J.D.P.; Lambert, S.M.; Ndifor, F.; Vyry W.N.A.; Henri, A.Z.P.; Chantal, M. 2009. Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. *African Journal of Biotechnology,* 8: 424-431.

Frodin, D.G. 2004. History and concepts of big plant genera. *Taxon*, 53: 753-776.

Gasparetto, A.; Cruz, A.B.; Wagner, T.M.; Bonomini, T.J.; Correa, R.; Malheiros, A. 2016. Seasonal variation in the chemical composition, antimicrobial and mutagenic potential of essential oils from *Piper cernuum*. *Industrial Crops and Products,* 95: 256-263.

Guimarães, E.F.; Carvalho-Silva, M.; Monteiro, D.; Medeiros, E. 2017. *Piperaceae. In*: Lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro. (http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB12735). Accessed on 12/19/2017.

Gogosz, A.M.; Boeger, M.R.T.; Negrelle1, R.R.B.; Bergo, C. 2012. Anatomia foliar comparativa de nove espécies do gênero *Piper* (Piperaceae). *Rodriguésia,* 63: 405-417.

Guerrini, A.; Sacchetti, G.; Rossi, D.; Paganetto, G.; Muzzoli, M.; Andreotti, E.; Tognolini, M.; Maldonado, M.E.; Bruni, R. 2009. Bioactivities of *Piper aduncum* L. and *Piper obliquum* Ruiz & Pavon (Piperaceae) essential oils from Eastern Ecuador. *Environmental Toxicology and Pharmacology,* 27: 39-48.

Hashim, N.A.; Ahmad, F.; Jani, N.A.; Susanti, D. 2017. In vitro Antioxidant, Antityrosinase, Antibacterial and Cytotoxicity Activities of the Leaf and Stem Essential Oil from *Piper magnibaccum* C. DC. *Journal of Essential Oil Bearing Plants,* 20: 223-232.

Hieu, L.D.; Thang, T.D.; Hoi, T.M.; Ogunwande, I.A. 2014. Chemical composition of essential oils from four Vietnamese species of *Piper* (Piperaceae*). Journal of Oleo Science,* 63: 211-217.

Li, R.; Yang, J.J.; Wang, Y.F.; Sun, Q.; Hu, H.B. 2014. Chemical composition, antioxidant, antimicrobial and anti-inflammatory activities of the stem and leaf essential oils from *Piper flaviflorum* from Xishuangbanna, SW China. *Natural Product Communications,* 9: 1011-1014.

Luz, A.I.R.; Silva, J.D.; Zoghbi, M.G.B.; Andrade, E.H.A.; Silva, M.H.I.; Maia, J.G.S. 2000. Volatile Constituents of Brazilian Piperaceae. Part 4. Essential Oil Composition of *Piper dactylostigmum, P. plurinervosum* and *P. vitaceum. Journal of Essential Oil Research,* 12: 94-96.

Machado, S.M.F.; Militão, J.S.L.T.; Facundo, V.A.; Ribeiro, A.; Morais, S.M.; Machado, M.I.L. 1994. Leaf Oils of Two Brazilian *Piper* Species: *Piper arboreum* Aublet var. *latifolium* (CDC) Yuncker and *Piper hispidum* Sw. *Journal of Essential Oil Research,* 6: 643-644.

Machado, N.S.O., 2007. *Estudo da anatomia foliar de espécies do gênero Piper L. (Piperaceae) no estado do Rio de Janeiro*. Doctoral thesis. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 103p.

Morais, S.M.; Facundo, V.A.; Bertini, L.M.; Cavalcanti, E.S.B.; Junior, J.F.A.; Ferreira, A.S.; Brito, E.S.; Neto, M.A.S.N. 2007. Chemical composition and larvicidal activity of essential oils from *Piper* species. *Biochemical Systematics and Ecology,* 35: 670-675.

Maia, J.G.S.; Andrade, E.H.A. 2009. Database of the Amazon aromatic plants and their essential oils. *Química Nova,* 32: 595-622.

Marques, A.M.; Fingolo, C.E.; Kaplan, M.A.C. 2017. HSCCC separation and enantiomeric distribution of key volatile

constituents of *Piper claussenianum* (Miq.) C. DC. (Piperaceae). *Food and Chemical Toxicology,* 109: 1111-1117.

Navickiene, H.M.D.; Morandim, A.A.; Alécio, A.C.; Regasini, L.O.; Bergamo, D.C.B.; Telascrea, M., *et. al*. 2006. Composition and Antifungal Activity of Essential Oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. *Química Nova,* 29: 467-470.

Oliveira, R.A.; Assis, A.M.A.D.; Silva, L.A.M.; Andrioli, J.L.; Oliveira, F.F. 2016. Chemical profile and antimicrobial activity of essential oil of *Piper ilheusense. Chemistry of Natural Compounds,* 52: 331-333.

Perigo, C.V.; Torres, R.B.; Bernacci, L.C.; Guimarães, E.F.; Haber, L.L.; Facanali, R.; Vieira, M.A.R.; Quecini, V.; Marques, O.M. 2016. The chemical composition and antibacterial activity of eleven *Piper* species from distinct rainforest areas in Southeastern Brazil. *Industrial Crops and Products,* 94: 528-539.

Quijano-Abril, M.A.; Callejas-Posada, R.; Miranda-Esquivel, D.R. 2006. Areas of endemism and distribution patterns for Neotropical *Piper* species (Piperaceae). *Journal of Biogeography,* 33: 1266-1278.

Rameshkumar, K.B.; Anu Aravind, A.P.; Mathew, P.J. 2011. Comparative Phytochemical Evaluation and Antioxidant Assay of *Piper longum* L. and *Piper chaba* Hunter Used in Indian Traditional Systems of Medicine. *Journal of Herbs, Spices & Medicinal Plants,* 17: 351-360.

Ruschel, D. 2004. *O Gênero Piper (Piperaceae) no Rio Grande do Sul*. Master's dissertation. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. 140p.

Saikia, A.K.; Sarma, S.K.; Strano, T.; Ruberto, G. 2015. Essential Oil from *Piper pedicellatum* C. DC. Collected in North-East India. *Journal of Essential Oil Bearing Plants,* 18: 314-319.

Salleh, W.M.; Kammil, M.F.; Ahmad, F., Sirat, H.M. 2015. Antioxidant and Anti-inflammatory Activities of Essential Oil and Extracts of *Piper miniatumi*. *Natural Product Communications,* 10: 2005-2008.

Santana, H.T.; Trindade, F.T.T.; Stabeli, R.G.; Silva, A.A.E.; Militão, J.S.L.T.; Facundo, V.A. 2015. Essential oils of leaves of *Piper* species display larvicidal activity against the dengue vector, *Aedes aegypti* (Diptera: Culicidae*). Revista Brasileira de Plantas Medicinais,* 17: 105-111.

Santos, P.R.D.; Moreira, D.L.; Guimarães, E.F.; Kaplan, M.A.C. 2001. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic forest. *Phytochemistry,* 58: 547-551.

Sperotto, A.R.M.; Moura, D.J.; Péres, V.F.; Damasceno, F.C.; Caramão, E.B.; Henriques, J.A.P.; Saffi, J. 2013. Cytotoxic Mechanism of *Piper gaudichaudianum* Kunth Essential Oil and its Major Compound Nerolidol. *Food and Chemical Toxicology,* 57: 57-68.

Trindade, A.P.F.; Velozo, L.S.M.; Guimarães, E.F.; Kaplan, M.A.C. 2010. Essential Oil From Organs of *Piper truncatum* Vell. *Journal of Essential Oil Research,* 22: 200-202.

Van Den Dool, H.; Kratz, P.D. 1963. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. *Journal Chromatography A*, 11: 463-471.

von Poser, G.L.; Rorig, L.R.; Henriques, A.T. 1994. Aromatic Plants from Brazil. III. The Chemical Composition of *Piper gaudichaudianum* Kunth and *P. mikanianum* (Kunth) Steudel Essential Oils. *Journal of Essential Oil Research,* 6: 337-340.

Woguem, V.; Maggi, F.; Fogang, H.P.; Tapondjoua, L.A.; Womeni, H.M.; Luana, Q. *et. al.* 2013. Antioxidant, antiproliferative and antimicrobial activities of the volatile oil from the wild pepper *Piper capense* used in Cameroon as a culinary spice. *Natural Product Communications,* 8: 1791-1796.

RECEIVED: 07/03/2018
ACCEPTED: 28/07/2018
ASSOCIATE EDITOR: Jorge David