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SHORT COMMUNICATION

First phytochemical description of essential oils from *Piper cachimboense* (Piperales, Piperaceae)

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ABSTRACT

Piper cachimboense is recorded only for the Amazon region of Brazil and Colombia, and the objective of this study was to report the first phytochemical assessment of the composition of the essential oils (EOs) from this species collected in the Amazon rainforest, in Novo Progresso, Pará State, Brazil. Samples of leaves were subjected to hydrodistillation in a Clevenger-type apparatus. The chemical identification was carried out by gas chromatography. The yield of oils was of 11.03 ± 5.94% for fresh leaves, and 1.07 ± 0.27% for dry leaves. The analysis showed 36 volatile compounds from fresh leaves and 49 from dried leaves. Main constituents in EOs of both fresh and dried leaves from *P. cachimboense* were (E)-caryophyllene, germacrene-D, γ-amorfene, δ-cadinene and apiole.

KEYWORDS: safrole, (*E*)-caryophyllene, germacrene-D, spathulenol, dillapiole

Primeira descrição fitoquímica de óleos essenciais de *Piper cachimboense* (Piperales, Piperaceae)

RESUMO

Piper cachimboense é registrada apenas para a região amazônica do Brasil e Colômbia, e o objetivo deste estudo foi relatar, pela primeira vez, a composição fitoquímica dos óleos essenciais (OEs) desta espécie coletada na floresta amazônica, em Novo Progresso/PA, Brasil. Amostras de folhas foram submetidas a hidrodestilação em aparelho tipo Clevenger. A identificação química foi realizada por cromatografia gasosa e o rendimento dos óleos foi de 11,03 ± 5,94% para as folhas frescas e de 1,07 ± 0,27% para as folhas secas. A análise mostrou 36 compostos voláteis para folhas frescas e 49 para folhas secas. Os constituintes principais dos OEs de folhas frescas e secas de *P. cachimboense* foram (*E*)-cariofileno, germacreno-D, γ-amorfeno, δ-cadineno e apiol.

PALAVRAS-CHAVE: safrol, (*E*)-cariofileno, germacreno-D, espatulenol, dilapiol

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Piperaceae Giseke, 1792, is a family of tropical and subtropical plants which occur in both hemispheres, including about 3,500 species, and the *Piper* genus is the largest, with more than 700 species, of which about 285 grow natively in Brazil and 192 are considered endemic (Monteiro and Guimarães 2009; Guimarães and Carvalho-Silva 2012).

Essential oils (EOs) from *Piper* are used in various sectors of the pharmaceutical, chemical and cosmetics industry (Andrade *et al.* 2009). However, although various Piperaceae produce essential oils in their leaves, only about 10% of *Piper* species have been chemically studied (Dyer *et al.* 2004). *Piper cachimboense* Yunck. (1966) has been recorded only in the Brazilian and Colombian Amazon, and the few publications about the species are restricted to distribution records (Bernal *et al*. 2016). Therefore, the objective of this study was to carry out the first phytochemical assessment of the composition of essential oils obtained from leaves of *P. cachimboense*.

Piper cachimboense leaves were harvested at the flowering stage (Figure 1) in the Florentino farm, in the municipality of Novo Progresso, Pará State, Brazil (7°06'56.31"S 55°24'22.19"W) at 210 masl, in March 2015. The species was identified and had vouchers deposited at Herbarium Tangará (TANG) of the State University of Mato Grosso, Campus of Tangará da Serra (UNEMAT/CUTS).

Samples of leaves (in triplicates of 100 g for fresh leaves, and of 50 g for dry leaves) were subjected to hydrodistillation in the Vegetable Ecophysiology Laboratory, at the Federal University of Paraná (UFPR). For the extraction of EOs from dry leaves, the materials were dried into forced air oven for 96 hours at 50 °C. For all oil extraction the leaves (fresh and dry) were placed in a glass flask (2 L) containing 1 L of distilled water. The water was boiled for 4 hours and the oil collected in a Clevenger-type apparatus. The volume measurement of EOs extracted from leaves was determined with the assistance of precision micropipettes (0-100 uL) and the yield was corrected to a dry basis after obtaining the constant weight of dried sub-samples in forced air oven at 65 °C. The yield was calculated based in the dry matter (DM), which is a standardized method that can be repeated at any time, without significant deviations (Santos *et al.* 2004).

Figure 1. Plant of *Piper cachimboense* (Note the characteristic fruit/inflorescence of this species) Photo: D. Krinski. This figure is in color in the electronic version.

Chromatographic analysis was performed in the Laboratory of Vegetable Ecophysiology and Laboratory of Natural Products and Chemical Ecology (LAPEQ), both at UFPR. The EOs were subjected to analysis by gas chromatography coupled to a flame ionization detector (HP-Agilent 7890A GC-FID) and by gas chromatography coupled to mass spectrometry (MS) (60–240 °C at 3 °C minutes rate) using a fused-silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm) coated with DB-5. The injector and detector temperatures were 280 °C. Hydrogen was used as carrier gas at a flow rate of 2.4 mL/minutes; injection was in the split mode (1:20), and the injection volume was 1.0 µL. MS spectra were obtained using electron ionization at 70 eV with a scan interval of 0.5 seconds and mass range from 40 to 550 *m/z*. The initial identification of components of the EOs was carried out by comparison with previously reported values of retention indices, obtained by co-injection of oil samples and C11–C24 linear hydrocarbons and calculated according to the equation of Van den Dool and Kratz (1963). Subsequently, the MS acquired for each component was matched with those stored in the Wiley/NBS mass spectral library of the GC-MS system and with other published mass spectral data (Adams 2007).

The yield of EOs were 11.03 ± 5.94 % for fresh leaves and 1.07 ± 0.27 % for dry leaves. The analysis showed 36 volatile compounds identified for fresh leaves and 49 for dried leaves, representing 78.62% and 82.51% of the total oil identified respectively.

According to Costa et al., (2005) the levels and chemical composition of essential oils of aromatic plants are influenced by several factors, such as the drying or not of the material used to obtain the oils. We observed in *P. cachimboense* a higher percentage of each compound for the oils obtained from dry leaves, although some compounds (δ-elemene, germacrene D, asaricin, γ-cadinene, globulol, dilapiolle, apiole and methyl linoleate) showed a higher percentage yield when using fresh leaves. A possibility for this result would be that in the process of drying the leaves, not only water but also part of these compounds would be lost. But, in general terms, both oils showed some similarity in the qualitative composition (Table 1).

Some compounds often found in essential Piperaceae oils, like safrole, apiol, spathulenol and dillapiole (Scott *et al*. 2008) were also present in *P. cachimboense*, as well as in other *Piper* species (e.g. Santos *et al.* 2001; Andrade *et al.* 2009; Cruz *et al.* 2011). These compounds are of economic interest. For example, safrole is the raw material for the synthesis of piperonal, which is used in the composition of perfumes (Barbosa *et al*. 2012). Besides the industrial

importance of its essential oils, *Piper* species have been used for many centuries in the traditional medicine for different purposes and many activities (Ghosh *et al*. 2014). Our data reinforce the accumulated knowledge that *Piper* species in general have a notable tendency to biosynthesize essential oils, independently of their natural habitats (Santos *et al.* 2001). This first report on the chemical composition of the essential oil of *P. cachimboense* shows that the phytochemical potential of many *Piper* species is still untapped.

Other studies are necessary to assess the seasonal effect on the overall yield, and the amount of major compounds of the essential oil of *P. cachimboense*. Considering that the leaves of *P. cachimboense* are easily harvested, the species is a good candidate for bioactivity testing of its oils regarding pest control in its occurrence region (Krinski 2013; Krinski 2015; Krinski *et al.* 2015; Krinski and Foerster 2017), as already known for other Piperaceae species (Krinski and Foerster 2016; Turchen *et al.* 2016; Sanini *et al.* 2017).

Table 1. Phytochemical composition of fresh and dried leaves of *Piper cachimboense* sampled at Novo Progresso, Pará State, Brazil. RI^c= retention index calculeted; RI^t= retention index tabulated (Adams 2007). Numbers for the relative area of leaves are means followed by the standard deviation.

Compounds identified	Retention Index		Relative area (%)	
	RI^c	RI^t	Fresh leaves	Dried leaves
1) α-pinene	932	932	0.06 ± 0.07	0.14 ± 0.06
2) α-terpinene	1014	1015	-	0.72 ± 0.17
3) p-cimene	1022	1022	-	0.67 ± 0.15
4) limonene	1024	1026	-	0.12 ± 0.04
5) γ-terpinene	1054	1056	-	1.90 ± 0.35
6) terpinolene	1086	1087	-	0.46 ± 0.08
7) linalol	1095	1100	-	0.16 ± 0.03
8) terpinen-4-ol	1174	1174	-	0.12 ± 0.01
9) piperitone	1249	1250	-	0.20 ± 0.01
10) safrole	1285	1284	0.27 ± 0.10	0.92 ± 0.14
11) δ-elemene	1335	1334	0.94 ± 0.10	0.52 ± 0.03
12) α-cubebene	1345	1346	0.19 ± 0.05	0.31 ± 0.03
13) α-copaene	1374	1371	0.85 ± 0.12	2.00 ± 0.15
14) β-elemene	1389	1388	1.08 ± 0.06	1.10 ± 0.03
15) α-gurjunene	1409	1403	-	0.17 ± 0.01
16) (*E*)-caryophyllene	1417	1413	4.65 ± 0.58	7.46 ± 0.39
17) β-gurjunene	1431	1423	0.63 ± 0.07	0.89 ± 0.03
18) aromadendrene	1439	1432	0.64 ± 0.09	1.39 ± 0.04
19) 6,9-guiadiene	1442	1437	0.13 ± 0.03	0.17 ± 0.00
20) α-humulene	1452	1446	1.34 ± 0.06	1.78 ± 0.06
21) *cis*-cadina-1(6),9-diene	1461	1452	1.00 ± 0.11	1.59 ± 0.05
22) γ-muurolene	1478	1472	-	3.98 ± 0.04
23) germacrene D	1480	1475	27.64 ± 2.29	6.31 ± 0.20
24) β-selinene	1489	1479	0.23 ± 0.01	1.92 ± 0.07
25) γ-amorfene	1495	1490	6.57 ± 0.98	6.88 ± 0.12
26) α-muurolene	1500	1493	-	1.53 ± 0.07
27) asaricin	1495	1495	1.93 ± 0.08	1.51 ± 0.02
28) β-bisabolene	1505	1502	0.70 ± 0.16	0.45 ± 0.02
29) γ-cadinene	1513	1508	3.10 ± 0.22	2.65 ± 0.03
30) δ-cadinene	1522	1519	6.19 ± 0.16	9.20 ± 0.04
31) *trans*-cadina-1,4-diene	1533	1526	0.62 ± 0.05	0.82 ± 0.02
32) α-cadinene	1537	1532	0.58 ± 0.03	0.59 ± 0.01
33) α-calacorene	1544	1536	0.19 ± 0.03	0.68 ± 0.01
34) elemicin	1555	1558	0.31 ± 0.04	0.93 ± 0.02
35) (*E*)-nerolidol	1561	1562	2.54 ± 0.94	3.37 ± 0.04
36) spathulenol	1577	1570	0.87 ± 0.06	4.65 ± 0.24

Table 1. Continued.

Compounds identified	Retention Index		Relative area (%)	
	RI[c]	RI[t]	Fresh leaves	Dried leaves
37) caryophyllene oxide	1582	1574	2.13 ± 0.33	3.50 ± 0.09
38) globulol	1590	1583	0.44 ± 0.08	0.29 ± 0.01
39) guaiol	1600	1585	0.54 ± 0.06	0.49 ± 0.02
40) rosifoliol	1600	1594	0.60 ± 0.05	0.71 ± 0.03
41) humulene epoxide II	1608	1599	0.17 ± 0.07	0.67 ± 0.03
42) 10-*epi*-γ-eudesmol	1622	1604	0.42 ± 0.09	0.46 ± 0.03
43) dilapiolle	1620	1607	1.29 ± 0.17	0.79 ± 0.03
44) *epi*-α-muurolol	1640	1622	1.41 ± 0.14	2.85 ± 0.19
45) cubenol	1645	1627	-	0.16 ± 0.01
46) α-cadinol	1652	1631	-	0.24 ± 0.03
47) apiole	1677	1647	5.68 ± 0.66	3.05 ± 0.26
48) (*E,Z*)-linalol de garnil	1987	2025	0.23 ± 0.11	0.49 ± 0.04
49) methyl linoleate	2095	2081	2.42 ± 0.23	0.54 ± 0.05
Total			78.62	82.51

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