

Leaf Essential Oils of Four *Piper* Species from the State of Ceará - Northeast of Brazil

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Os óleos essenciais obtidos por hidrodestilação das folhas de quatro espécies de *Piper* (*P. arboreum*, *P. crassinervium*, *P. dilatatum* and *P. tuberculatum*), coletadas no estado do Ceará, foram analisados por CG-EM. Os rendimentos dos óleos, calculados sobre o peso do material fresco, variaram entre 0,03 – 0,11% (p/p). Os óleos foram caracterizados pela presença de mono- e sesquiterpenos, exceto o óleo de *P. arboreum*, no qual foram identificados apenas sesquiterpenos. β -Elemeno (0,58-3,03%), (*E*)-cariofileno (2,71-37,78%), germacreno D (3,43-11,81%), biciclogermacreno (2,83-25,07%) e δ -cadineno (0,52-2,44%) foram detectados em todas as amostras analisadas. Os monoterpenos majoritários identificados foram α -pineno (11,27%), β -pineno (20,01%), 1,8-cineol (10,81%) e linalol (28,61%) para *P. crassinervium*, e α -felandreno (22,53%) e Δ -3-careno (10,20%) para *P. dilatatum*. Biciclogermacreno (25,03 e 25,07%) para *P. arboreum* e *P. dilatatum*, (*E*)-nerolidol (11,12%) para *P. arboreum*, germacreno D (11,81%) para *P. tuberculatum* e (*E*)-cariofileno (10,26 e 37,78%) para *P. dilatatum* e *P. tuberculatum*, foram os principais sesquiterpenos. Este trabalho descreve, pela primeira vez, a composição química dos óleos essenciais de *P. crassinervium* e *P. tuberculatum*.

The essential oils, obtained by hydrodistillation, from leaf samples of four *Piper* species (*P. arboreum*, *P. crassinervium*, *P. dilatatum* and *P. tuberculatum*), harvested at the State of Ceará, were analyzed by GC-MS. The oil yields ranged from 0.03 to 0.11% (w/w), on fresh weight basis. The oils were characterized by mono- and sesquiterpenes, except the oil of *P. arboreum* to which only sesquiterpenes were identified. β -Elemene (0.58-3.03%), (*E*)-caryophyllene (2.71-37.78%), germacrene D (3.43-11.81%), bicyclogermacrene (2.83 and 25.07%) and δ -cadinene (0.52-2.44%) were detected in all oil samples. The major identified monoterpenes were α -pinene (11.27%), β -pinene (20.01%), 1,8-cineole (10.81%) and linalool (28.61%) for *P. crassinervium* and, α -phellandrene (22.53%) and Δ -3-carene (10.20%) for *P. dilatatum*. The major sesquiterpenes were bicyclogermacrene (25.03 and 25.07%) for *P. arboreum* and *P. dilatatum*, (*E*)-nerolidol (11.12%) for *P. arboreum*, germacrene D (11.81%) for *P. tuberculatum* and, (*E*)-caryophyllene (10.26 and 37.78%) for *P. dilatatum* and *P. tuberculatum*. To the best of our knowledge this is the first report about the chemical composition of the leaf essential oils of *P. crassinervium* and *P. tuberculatum*.

Keywords: *Piper arboreum*, *Piper crassinervium*, *Piper dilatatum*, *Piper tuberculatum*, essential oils

Introduction

The genus *Piper* (Piperaceae) has been recently revised.¹ With approximately 700 species, represented by herbs, shrubs and trees it is widely distributed in the tropical and subtropical regions of the terrestrial globe. Several plants of this genus are largely used in folk medicine in several parts of the world and have been reported to produce compounds

with diverse biological and pharmacological properties.²⁻⁸ Many *Piper* species are aromatic and as a consequence the chemical composition of the essential oils of several of them has been the subject of incessant studies, revealing a diverse range of volatile components, including monoterpenes, sesquiterpenes, arylpropanoids, aldehydes, ketones and long chain alcohols.⁹⁻¹⁶

Pursuing the determination of the chemical composition of essential oils from aromatic and medicinal plants from Northeast of Brazil flora, especially from the State of Ceará,

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we are reporting now the results of the investigation of four *Piper* species: *P. dilatatum* L.C. Rich, *P. arboreum* Aubl., *P. tuberculatum* Jacq. and *P. crassinervium* Kunth. Although the non-volatile constitution of all four species have already been reported,^{2,6,17-19} the essential oils of *P. tuberculatum* and *P. crassinervium* are being reported for the first time. During referee assessment of this paper a report of the essential oils from eleven different plant species, including *P. crassinervium*, came out in the literature.²⁰

Results and Discussion

The results for the leaf essential oil analysis of four *Piper* species are described in Table 1. A total of 52 volatile constituents were identified, accounting for 88.49 – 98.62% of the chemical composition of the correspondent oils. All oils showed some similarity in the qualitative composition, but they differ significantly from a quantitative point of view. The essential oils of *P. arboreum* and *P. tuberculatum* are characterized by the presence of sesquiterpenes, 88.49 and 81.07%, respectively; while a high percentage of monoterpenes was detected in the oil of *P. crassinervium* (74.55%). On the other hand, a similar contribution of monoterpenes (42.14%) and sesquiterpenes (54.19%) was observed to the oil of *P. dilatatum*. As can be seen from Table 1, monoterpenes were not detected in the essential oil of *P. arboreum*, to which the most significant constituents were the sesquiterpenes bicyclogermacrene (25.07%), (*E*)-nerolidol (11.12%) and (*E*)-caryophyllene (8.35%). Previous studies on leaf essential oils of what seems to be three varieties of *P. arboreum* have been reported and significant differences were found in their chemical compositions, although all them were dominated by sesquiterpenes.^{10,11,15} The leaf essential oil of *P. arboreum* Aublet var. *latifolium* (C.DC) Yuncker from Rondônia, was characterized by an higher content of germacrene D (72.87%),¹⁰ while in the leaf oil of *P. arboreum* var. *arboreum* Yunck. from Rio de Janeiro, the most abundant constituents were γ -eudesmol (14.61%) and α -eudesmol (12.21%), followed by bulnesol (8.13%) and (*E*)-caryophyllene (6.40%).¹⁵ On the other hand, in the study of *P. arboreum* var. *latifolium* oil from Panama, δ -cadinene (25.80%) and α -copaene (7.40%) were the main compounds.¹¹ Comparison of those major constituents for all *P. arboreum* oils from different origins revealed that all components are present in the oil from Rio de Janeiro, just two of them (germacrene D and α -copaene) are present in the oil from Rondônia and, that the same compounds (germacrene D, (*E*)-caryophyllene, δ -cadinene and α -copaene) are qualitatively but not quantitatively, present in both oils from Ceará and Panama. In the essential oil of

P. dilatatum, from Ceará, α -phellandrene (22.53%) and Δ -3-carene (10.20%) were the major monoterpene hydrocarbons, while bicyclogermacrene (25.03%) and (*E*)-caryophyllene (10.26%) were the main sesquiterpene hydrocarbons. Earlier investigation of the leaf essential oil of *P. dilatatum* from Rio de Janeiro, showed that the monoterpenes myrcene (41.70%) and α -pinene (17.70%) were the major components.¹⁵ The major mono- and sesquiterpenes of the oil from Ceará are absent, or in very low percentage, in the oil from Rio de Janeiro. The same thing happens with the major monoterpenes of the oil from Rio de Janeiro. The oil of *P. crassinervium*, rich in monoterpenes, was characterized by a high content of linalool (28.61%), β -pinene (20.01%), α -pinene (11.27%) and 1,8-cineole (10.81%). Recently, the essential oil of *P. crassinervium* from Ecuador has been examined and was found to be rich in monoterpenes, including α -pinene (15.17%) and β -pinene (10.0%), in accordance with our analysis, but also rich in limonene (26.6%) and α -terpinene (7.79%),²⁰ not present in the oil from Ceará. All these facts would be maybe attributed to the edafoclimatic conditions related to the different environments in which the plants grown. The most significant constituents of the oil of *P. tuberculatum* were the sesquiterpenes (*E*)-caryophyllene (37.78%) and germacrene D (11.81%), while α -pinene (4.06%) and β -pinene (4.51%) were the main constituents of the monoterpene fraction. Unfortunately no other source of *P. tuberculatum* oil was found in the literature for comparison.

Of the 52 identified compounds, β -elemene, (*E*)-caryophyllene, germacrene D, bicyclogermacrene and δ -cadinene were detected in all four oil samples, while α -pinene, β -pinene, myrcene, δ -elemene, α -humulene, elemol, (*E*)-nerolidol and β -eudesmol were found in three of them. As can be observed from our findings, as well as from other analysis on essential oil from *Piper* species,^{10-13,15,16} there is a notable tendency of those plants to biosynthesize sesquiterpenes, independent of their natural habitats.

Experimental

Plant material

The leaves of *P. dilatatum* (# 33.821), *P. crassinervium* (# 33.877) and *P. arboreum* (# 33.872) were harvested at the flowering stage from Guaramiranga Mountain (Ceará State, Brazil), in June 2004. *P. tuberculatum* (# 12985) was harvested from Horto de Plantas Mediciniais Prof. Francisco José de Abreu Matos - Universidade Federal do Ceará, in July 2004. All species were harvested from 9 to

Table 1. Chemical composition of four *Piper* species^a

Volatile components ^b	Relative area (%)					
	RI ^c	RI ^d	Pa	Pc	Pd	Pt
1. α -pinene	947-952	939	-	11.27	1.40	4.06
2. sabinene	977	975	-	-	-	0.45
3. β -pinene	977-979	979	-	20.01	0.88	4.51
4. myrcene	987-990	991	-	0.93	0.78	0.29
5. α -phellandrene	1001	1003	-	-	22.53	-
6. <i>p</i> -cymene	1015	1025	-	-	0.78	-
7. sylvestrene	1019	1031	-	-	-	0.79
8. Δ -3-carene	1019	1031	-	-	10.20	-
9. 1,8-cineole	1020	1031	-	10.81	-	-
10. (<i>Z</i>)- β -ocimene	1027	1037	-	-	0.68	-
11. (<i>E</i>)- β -ocimene	1037-1039	1050	-	-	4.89	0.90
12. linalool	1090	1097	-	28.61	-	-
13. borneol	1150	1169	-	0.79	-	-
14. α -terpineol	1178	1189	-	2.13	-	-
15. δ -elemene	1331-1335	1338	0.75	0.57	0.90	-
16. α -copaene	1373-1374	1377	1.86	-	-	0.98
17. β -cubebene	1389	1388	1.11	-	-	-
18. β -elemene	1390-1393	1391	2.70	0.58	1.40	3.03
19. (<i>E</i>)-caryophyllene	1417-1025	1419	8.35	2.71	10.26	37.78
20. β -copaene	1428	1432	0.92	-	-	-
21. aromadendrene	1438	1441	0.43	-	-	-
22. α -humulene	1451-1454	1455	1.83	-	0.86	2.82
23. <i>allo</i> -aromadendrene	1459-1461	1460	-	-	1.14	0.41
24. γ -muurolene	1479	1480	0.52	-	-	-
25. germacrene D	1481-1483	1485	5.29	3.43	4.23	11.81
26. β -selinene	1488	1490	-	-	-	3.22
27. bicyclogermacrene	1498-1500	1500	25.07	2.83	25.03	4.42
28. α -muurolene	1503-1504	1500	1.29	-	-	0.30
29. germacrene A	1506	1509	0.47	-	-	0.60
30. δ -amorphene	1509	1512	-	-	1.10	-
31. γ -cadinene	1515	1514	-	-	-	0.24
32. cubebol	1518	1515	1.14	-	-	-
33. δ -cadinene	1025-1027	1523	2.44	0.52	0.63	0.64
34. elemol	1551-1552	1550	1.48	0.68	-	2.15
35. germacrene B	1556	1561	-	0.99	0.92	-
36. (<i>E</i>)-nerolidol	1568-1570	1563	11.12	-	6.21	3.81
37. germacrene D-4-ol	1576	1576	-	-	-	1.64
37. spathulenol	1578	1578	2.75	-	-	-
38. caryophyllene oxide	1581	1583	-	0.59	-	2.74
39. viridiflorol	1583-1591	1593	0.62	0.62	-	-
40. guaiol	1597-1599	1601	-	0.81	-	0.43
41. 5- <i>epi</i> -7- <i>epi</i> - α -eudesmol	1606	1608	1.42	-	-	-
42. 10- <i>epi</i> - γ -eudesmol	1618	1624	4.26	-	-	-
43. eremoligenol	1627-1635	1631	5.22	0.91	-	-
44. γ -eudesmol	1630	1632	-	1.08	-	-
45. <i>epi</i> - α -cadinol	1639	1640	-	0.90	-	-
46. <i>epi</i> - α -muurolol	1640	1642	-	-	0.70	-
47. α -muurolol	1645	1646	0.59	-	0.81	-
48. β -eudesmol	1645-1647	1651	2.60	3.11	-	1.50
49. α -eudesmol	1651	1654	-	3.74	-	1.50
50. valerianol	1651	1658	3.51	-	-	-
51. 7- <i>epi</i> - α -eudesmol	1655	1664	0.75	-	-	-
52. bulnesol	1663	1672	-	-	-	1.05
Terpenoids classes						
Monoterpene hydrocarbons			-	32.21	42.14	11.00
Oxygenated monoterpenes			-	42.34	-	-
Sesquiterpene hydrocarbons			53.03	11.63	46.47	66.25
Oxygenated sesquiterpenes			35.46	12.44	7.72	14.82
Total			88.49	98.62	96.43	92.07

^aPa = *P. arboreum*; Pc = *P. crassinervium*; Pd = *P. dilatatum*; Pt = *P. tuberculatum*; ^bCompounds are listed in order of their elution from a DB-5 column; ^cRI = retention indices relative to C₈ – C₂₆ n-alkanes; ^dRI = retention indices from literature.¹⁸

11 am. and the voucher specimens have been deposited at the Herbarium Prisco Bezerra (EAC), Universidade Federal do Ceará.

Isolation of the essential oils

Fresh leaf samples of all species were subjected to hydrodistillation for 2 hours in a Clevenger-type apparatus. The isolated essential oils were dried over anhydrous sodium sulfate and, after filtration, maintained under refrigeration before analysis. The yields (m/m) of the oils were calculated based on the fresh weight of the plant materials: *P. dilatatum* (600 g, 0.07%), *P. crassinervium* (290 g, 0.11%), *P. arboreum* (700 g, 0.07%) and *P. tuberculatum* (660 g, 0.03%).

GC-MS conditions

GC-MS analysis was carried out on a Hewlett-Packard Model 5971 GC/MS using a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness); helium as the carrier gas, flow rate of 1 mL min⁻¹ and with split ratio 1:30. The injector temperature and detector temperature was 250 °C and 200 °C, respectively. The column temperature was programmed from 35 °C to 180 °C at 4 °C min⁻¹ and then from 180 °C to 250 °C at 10 °C min⁻¹. Mass spectra were recorded from 30 – 450 *m/z*.

Compound identification

The volatile components were identified by comparison of their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library and other two computer libraries MS searches using retention indices as a preselection routine.^{21,22} The identifications were confirmed by comparison of the fragmentation pattern and their retention indices with those reported in the literature.^{23,24}

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