Chemical characterization of seven *Piper* species (Piperaceae) from Federal District, Brazil, based on volatile oil constituents

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ABSTRACT: Chemical characterization of seven *Piper* species (Piperaceae) from Federal District, Brazil, based on volatile oil constituents. Sixteen *Piper* species has been described in the Federal District area, with some species containing aroma due the presence of essential oils. Among them, leaf samples of seven *Piper* species were collected at Fazenda Sucupira, Embrapa Genetic Resources and Biotechnology. The essential oil was extracted by hydrodistillation and analyzed by gas chromatography and mass spectroscopy to characterize the chemical composition of each species. Preliminaries results indicate a high yield of essential oil of *Piper xylosteoides* Kunth, with 1.78% of essential oil in relation to the dry weight of the samples. Compounds like β-pinene, myrcene, limonene, caryophyllene, γ-muurolene, nerolidol, spathulenol, viridiflorol and caryophyllene oxide are presented in almost *Piper* species studied. The major compounds are α-pinene in *Piper amalago, cis*-β-ocimene e β-caryophyllene in *P.dilatatum*, α-pinene e β-pinene in *P. ovatum,* myrcene in *P. xylosteoides*, b-pinene in *P. hispidum*, bicyclogermacrene in *P.arboreum,* α-pinene, β-pinene e caryophyllene oxide in *P. tectonifolium*.

Key words: *essential oil, chemical composition.*

INTRODUCTION

The Brazilian Cerrado contains a rich flora of medicinal use. The intense collection from wild species and the expansion of agriculture have been contributing to the loss of genetic variability of medicinal and aromatic species in the Cerrado. The family Piperaceae, well established in tropical areas, contain several aromatic species used in the Brazilian traditional medicine (BERNARD *et al*, 1995). Piperaceae has five genera and 1,400 species. *Piper* L. is the most representative genus with 700 species. In the Federal District area, sixteen *Piper* species have been described (Carvalho-Silva & Cavalcanti, 2002), some containing aroma due the presence of essential oils. The main goal of this work is to identify the main essential oil constituents of seven *Piper* species from Federal District, Brazil.

MATERIAL AND METHOD

Leaf samples of *Piper amalago*, *P. dilatatum*, *P. ovatum*, *P. arboreum*, *P. hispidum*, *P. xylosteoides*, and *P. tectonifolium* were collected at Fazenda Sucupira, Embrapa, Brasília, in December, 2003 (Table 1). Voucher specimens were identified and stored at Embrapa Genetic Resources and Biotechnology herbarium (CEN). Leaves were harvested, weighted, placed in a paper bag, and dried in a forced-air drier at 38ºC for 3 days for oil analysis. The essential oil was extracted by hidrodistillation in a modified Clevenger apparatus, in a 2 L flask during one and a half hour.

The oil composition was analyzed in an Agilent 6890N gas chromatograph fitted with a HP-5 (25m X 0.32mm X 0.25mm) capillary column. The oven temperature was programmed from 60ºC to 240ºC at 3ºC/min, and hydrogen was used as carrier gas (1.4 mL/min). 1.0mL of a 10% solution of the oil in dichlormethane was injected in split mode (1:100; injector at 250ºC).

Mass spectra were obtained in an Agilent 5973N system operating in electron impact mode (EIMS) at 70 eV, coupled to an Agilent 6890 gas chromatograph fitted with a HP-5 MS column (30m X 0.25mm X 0.25mm), using the same injection procedure and oven temperature program as above. Helium was the carrier gas, at 1.0 mL/min. The identification was based on the mass spectra of the compounds compared with the data in Wiley 6th ed. library and by their calculated retention indices (RI) compared with literature data.

RESULT AND DISCUSSION

This is the first report on *Piper* species from Federal District, Brazil. Among seven *Piper* species

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studied, *Piper xylosteoides* (1.78% of essential oil on dry weigh basis) showed the highest percentage of essential oil based on dry weight basis. The other species presented an essential oil content varying from 0.2 to 0.5% (Table 1).

Essential oil from *Piper* species leaves presented around 18 and 34 constituents. Table 2 illustrates the major constituents in the seven *Piper* species. Compounds like β-pinene, myrcene, limonene, caryophyllene, γ-muurolene, nerolidol, spathulenol, viridiflorol and caryophyllene oxide (Table 2) are presented in most *Piper* species.

Some compounds are unique for specific *taxa*, such as borneol (5,6%) in *P. amalago*; *trans*-â-ocimene (3.2%) and *cis*-â-ocimene (18.7%); in *P. hispidum*, â-phellandrene (3.47%) in *P. xylosteoides*; and germacrene B (2.77%) in *P. dilatatum*.

Many of these essential oil constituents are used in the industry. α-pinene, a common constituent of several volatile oils, is used in the manufacturing of camphor, insecticides, solvents and perfume basis. Limonene is a solvent, wetting and dispersing agent. Borneol is primarelly used in the in perfumery industry and incense making (Windholz, 1983).

Myrcene is the major constituent of the volatile oil from *P. xylosteoides*, which has also presented the highest yield (30%). Myrcene is largely used in food and cosmetic industry. Also, it is known as analgesic and for its antimicrobial properties.

The seven species of *Piper* has shown an enormous level of chemical diversity, considering that all species were collected in the same location. Further studies on population variability will be necessary to better understand the volatile oil production of this species.

Although we verify a chemical profile for essentail oils of Piper species from Federal District, differences on chemical constituents composition is observed when same species were harvested from other biome, as described for *Piper arboretum*, *P. dilatatum* e *P. hispidum* in Mata Atlantica (Santos, *et al.* 2001*)*.

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TABLE 1. Leaf essential oil content of nine *Piper* species from Federal District, Brazil

Species	Leaf Fresh weight (g)	Leaf Dry weight (g)	Essential Oil Content (ml)	Essential Oil Yield %
P. amalago L.	374	90	0,5	0,56
P. arboreum Aubl. subsp *arboreum*	560	155	0,5	0,32
P. hispidum C.CD.	497	124	0,4	0,32
P. dilatatum L.C.Rich.	298	56	0,2	0,36
P. ovatum Vahl.	246	59	0,2	0,34
P. tectonifolium Kunth.	768	161	0,4	0,25
P. xylosteoides Kunth.	271	84	1,5	1,78

TABLE 2. Major constituents of seven *Piper* species essential oil from Federal District, Brasil.

Volatile oil constituents [a]	RT [b]	*Piper* species [c]						
		Am	Di	Ov	Ar	Hi	Xy	Te
		(relative percentage of total volatile oil)						
α-Pinene	6.5	30.5	0.5	23.1	-	9.0	2.4	12.9
Camphene	6.9	8.9	-	0.3	-	-	2.6	0.6
Sabinene	7.6	0.3	0.9	1.7	-	-	1.7	0.3
β-Pinene	7.7	1.3	-	14.2	-	19.7	0.4	8.8
Myrcene	8.0	1.6	0.7	1.7	-	0.5	31.0	-
δ-3-Carene	8.7	-	-	-	0.9	7.4	-	-
α-Terpinene	9.0	-	-	-	-	-	11.3	-
p-Cimene	9.2	2.1	0.7	3.2	-	1.2	12.4	-
β-Phelandrene	9.4	-	-	-	-	-	2.1	-
Limonene	9.4	6.8	2.9	0.3	-	1.2	-	0.3
trans-β-Ocimene	9.6	-	3.3	-	-	-	-	-
cis-β-Ocimene	10.0	-	19.7	-	-	-	-	-
γ-Terpinene	10.4	-	0.3	-	-	-	26.1	-
Borneol	14.8	5.7	-	-	-	-	-	-
Mirtenal	16.2	-	-	-	-	-	-	2.4
Bornyl acetate	20.2	3.4	-	-	-	1.0	2.3	0.8
α-Copaene	22.0	1.5	-	0.7	-	-	-	2.1
Neryl acetate	23.8	-	-	-	-	2.7	-	-
β-Elemene	24.9	-	2.2	1.7	0.6	-	-	2.7
β-Caryophyllene	26.1	0.6	11.4	5.3	3.0	-	0.5	-
α-Humulene	27.6	-	1.7	4.1	0.5	-	-	0.3
γ-Muurolene	28.6	0.5	-	0.3	0.3	3.1	-	0.6
Germacrene-D	28.8	-	8.9	10.3	1.5	-	2.8	-
Epicubebol	29.4	-	-	10.7	-	-	-	1.2
Bicyclogermacrene	29.5	-	8.8	2.6	12.1	-	0.6	-
Cubebol	30.2	-	0.8	3.2	0.9	2.0	-	-
δ-Cadinene	30.2	4.7	0.8	2.9	0.7	-	-	1.3
Elemol	31.6	0.8	2.4	0.7	1.5	-	-	-
Germacrene B	31.9	-	2.8	-	-	-	-	-
Nerolidol	32.2	-	2.8	2.6	2.3	-	-	1.4
Spathulenol	32.8	4.2	6.5	1.0	8.4	6.2	0.6	4.6
Caryophyllene Oxide	33.0	2.3	5.3	1.3	10.2	4.0	-	10.9
Viridiflorol	33.4	2.9	-	0.5	-	1.4	-	2.9
Humulene epoxide II	34.1	-	1.0	-	1.0	1.6	-	2.8
NI [d]	34.6	-	-	-	11.6	-	-	-
γ-Eudesmol	35.0	-	-	-	6.7	1.6	-	-
NI [d]	35.3	-	-	-	13.5	-	-	-
β-Eudesmol	35.7	-	1.6	0.9	-	3.9	-	-
α-Cadinol	35.8	1.2	1.4	0.9	-	6.9	-	-
Total (%)		79.0	87.3	93.9	75.8	73.4	96.8	56.8

[a] Listed in order of retention time (min); [b] RT = Retention Time.

[c] Am=*P. amalago*; Di=*P. dilatatum*; Ov=*P. ovatum*; Ar=*P. arboreum*; Hi=*P. hispidum*; Xy=*P. xylosteoides*; Te=*P. tectonifolium*, NI[d]=not identified