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Seasonal variation in the essential oil of *Pilocarpus microphyllus* Stapf.

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ABSTRACT

The essential oils of the leaves and fine stems of *Pilocarpus microphyllus*, collected on iron mineralized soil of the Serra de Carajás, Southeast of Pará State, Brazil, during the rainy and dry seasons, were obtained by hydrodistillation and analyzed by GC-MS. The main identified compounds were 2-tridecanone, β-caryophyllene, 2-pentadecanone, caryophyllene oxide and germacrene D. Their percentage contents varied with the season, the greater values having been detected mainly in the rainy season. For 2-tridecanone and β-caryophyllene the higher values were observed in the fine stem oils for the former, and in the leaf oils for the latter. For 2-pentadecanone, caryophyllene oxide and germacrene D they were also in the leaf oils. In general, the leaf oils were very distinguishable from those of fine stem oils, even in the same specimen.

Key words: *Pilocarpus microphyllus*, Rutaceae, jaborandi, essential oil composition, 2-tridecanone, 2-pentadecanone, sesquiterpenes.

INTRODUCTION

The genus *Pilocarpus* (Family Rutaceae) comprises about 13 species, 7 subspecies and 8 varieties occurring throughout the neotropics, but completely absent from the Amazon Basin (Kaastra 1982). Most of them are distributed from the South of Pará State to Northeast Brazil where they are used as medicinals. The species *Pilocarpus microphyllus* Stapf. is known as ''jaborandi'' or ''jaborandi-do-maranhão'' in the East Amazon and its leaves are used as diuretic, sudorific, digestive, sialogogue, hair tonic, for eye inflammation, and mainly for industrial extraction of pilocarpine, an imidazole alkaloid with cholinergic activiy (Sousa et al. 1991, Pio Corrêa 1984, Maia et al. 2001).

The oils of the species of *P. racemosus* (Guadeloupe jaborandi), *P. microphyllus* (Maranham jaborandi) and *P. spicatus* (Aracati jaborandi) were previously reported as containing an appreciable content of 2-nonanone (Guenter 1972). Later on these data were reviewed after the analysis of the steam distilled oils from *P. jaborandi* (Pernambuco jaborandi), *P. microphyllus* and *P. pauciflorus* (subspecies of *P. spicatus*) (Craveiro et al. 1979, 1981). According to this study the main compounds found in the oil of *P. jaborandi* were limonene and 2-undecanone; in the oil of *P. microphyllus* were caryophyllene and 2-tridecanone; and in the oil of *P.*

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pauciflorus were sabinene and 2-undecanone. The essential oils of some plant parts of two specimens of *P. trachyllophus* (Ceará jaborandi) was also analyzed and the major identified constituents were β-caryophyllene, germacrene D, 2-tridecanone, spathulenol and 2-pentadecanone (Andrade-Neto et al. 1995, 2000).

Now, we wish to report the chemical composition of the essential oils of three specimens of *P. microphyllus* growing wild in the Mineral Province of the Serra de Carajás, Southeast of Pará State, Brazil, in an iron mineralized soil, during the rainy and dry seasons. The work was part of a project to identify biogeochemical markers of mineral soils in the Amazon, based on essential oil analysis (Taveira 1991).

MATERIALS AND METHODS

Material and extraction of volatile constituents

The leaves (F) and fine stems (G) of four specimens of *P. microphyllus* were collected in the N-1 area (iron mine) belonging to Companhia Vale do Rio Doce (CVRD), in the Serra de Carajás, Southeast of Pará State, Brazil, during the rainy (March/April) and dry (October/November) season. Voucher specimens (#131.456) have been kept at the herbarium of Museu Paraense Emílio Goeldi, in the city of Belém, Pará State, Brazil. The plant parts of samples 131F, 131G, 180F, 180G, 199F and 199G were air-dried for 48 hours, mill powdered to furnish 200 g each, and separately hydrodistilled for 4h using a Clevenger-type apparatus. The aerial part (leaves and fine stems) of PPCE138 sample was air-dried for 5 days, mill powdered (200g) and submitted to hidrodestillation at the same condition. The distilled oils were dried over anhydrous sodium sulfate and the percentage contents were calculated on basis of the dry weight of plant material. The plant collection site and the oil yields are listed in Table I.

Analysis of the volatile constituents

Analyses of the volatile compounds were performed on two GC/MS instruments: a (1) HP 5988 and a (2) Finnigan Mat INCOS XL, under the following conditions: (1) WCOT SE-54 (25m $\times$ 0.32mm; 0.25μm film thickness) and (2) WCOT DB-5ms (30m $\times$ 0.25mm; 0.25μm film thickness) fused silica capillary columns; temperature programmed: (1) 50°C-180°C (4°C/min) and 180°C-250°C (2°C/min) and (2) 60°C-240°C (3°C/min); injector temperature: 220°C; carrier gas: He, adjusted to a linear velocity of 32cm/sec (measured at 100°C); split flow was adjusted to give a 20:1 ratio; injection type: splitless (1μl, of a 1:1000 hexane solution); EIMS: electron energy, 70eV; ion source temperature and connection parts: 180°C.

Individual components were identified by comparison of both mass spectrum and their GC retention data with those of authentic compounds previously analyzed and stored in the data system. Other identifications were made by comparison of mass spectra with those in the data system libraries and cited in the literature (Adams 1995). The retention indices (RI) were calculated for all volatile constituents using an *n*-alkanes homologous series at the same GC conditions.

RESULTS AND DISCUSSION

Based on GC-MS analysis 27 compounds were identified in the oil samples of *P. microphyllus*. These data are listed in Table II. The main compounds were 2-tridecanone, β-caryophyllene, 2-pentadecanone, caryophyllene oxide and germacrene D. Their percentage contents varied with the season, the greater values having been found mainly in the rainy period. The higher contents of 2-tridecanone and β-caryophyllene were observed in the fine stem oils (131G: 36.0%; 199G: 64.1%) for the former, and in the leaf oils (131F: 40.6%; 199F: 29.2%) for the latter. The higher contents of 2-pentadecanone was in the leaf oil (131F: 28.1%); and for caryophyllene oxide (199F: 15.4%) and germacrene D (131F: 10.5%; 199F; 8.4%) were in the leaf oils. In general, the leaf oil composition was very distinct from those of the fine stem oils, even for the same specimen, as observed for the samples 180F and 180G,

TABLE I

Collection data and oil yields of the samples of *Pilocarpus microphyllus*.

Reg. No.	Plant Part	Season	Collection Site	Vegetation Type	Oil Yield (%)
131F	Leaves	Rainy	N-1 area, iron mine	Canga	0.4
131G	Fine stems				0.2
180F	Leaves	Dry	N-1 area, iron mine	Canga	0.3
180G	Fine stems				0.2
199F	Leaves	Rainy	N-1 area, iron mine	Canga	0.3
199G	Fine stems				0.2
PPCE138	Leaves and fine stems	Dry	N-1 area, iron mine	Canga	0.2

Canga – Top soil made up mostly of iron oxides.

collected in the dry season. Analyzing the principal methyl ketones, probably originated from the same biosynthetic pathway, it was observed that the pair 2-tridecanone:2-pentadecanone presented an inversion of their percentage contents, according with the analyzed plant part. For example, the values for 2-tridecanone and 2-pentadecanone in the 199F leaf oil were 2.4% and 14.2%, respectively, contrasting with those observed in the 199G fine stem oil, that were 64.1% and 18.9%, respectively. The same inversion can be observed for the other samples (131F:131G and 180F:180G). The leaf and fine stem oils obtained from the specimen PPCE138, collected during the dry season, presented the higher percentage content for 2-tridecanone (88.1%). This noteworthy value is attributed to a longer sample drying time when other more volatile compounds were probably lost.

In the present study it was not possible to identify the monoterpenes limonene, β-ocimene and α-pinene, as well as the hydrocarbon 3,7,7-trimethylbicyclo[3,1,1]-2-heptene, previously described for *P. microphyllus* oil (Craveiro et al. 1979, 1981). On the other hand, the chemical composition reported for the *P. trachyllophus* oil (Andrade-Neto et al. 1995, 2000) showed some similarities with our analyses, except for the conspicuous presence of spathulenol, in the place of caryophyllene oxide, found by us in the oil of *P. microphyllus*.

These results are based on single samples at each collection site and do not take into account the within site variation. However, the chemical composition of the analyzed essential oils showed qualitative and quantitative variation by the influence of local environmental conditions of soil and seasonal period of collections. These findings have ecological and taxonomic significance for the species application as biogeochemical or chemotaxonomic markers.

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RESUMO

Os óleos essenciais das folhas e galhos finos de *Pilocarpus microphyllus*, coletado em solo mineralizado com ferro, na Serra de Carajás, Sudeste do Pará, Brasil, durante as estações chuvosa e seca, foram obtidos por hidrodestilação e analisados por GC-MS. Os principais compostos identificados foram 2-tridecanona, β-cariofileno, 2-pentadecanona, óxido de cariofileno e germacreno D. Seus teores percentuais variam com a estação, embora os maiores valores tenham sido detectados principalmente na estação chuvosa. Para 2-tridecanona e β-cariofileno os valores mais expressivos foram observados nos óleos de galhos finos, para o primeiro, e nos óleos das folhas, para o útimo. Para 2-pentadecanona, óxido de cariofileno e germacreno

TABLE II

Volatile constituents (%) of the essential oils of *Pilocarpus microphyllus*.

Constituents	RI*	Rainy season		Dry season		Rainy season		Dry season
		Sample 131F	Sample 131G	Sample 180F	Sample 180G	Sample 199F	Sample 199G	Sample PPCE138
δ-Elemene	1336	–	–	–	0.8	–	–	–
α-Cubebene	1348	–	–	0.3	2.0	–	–	–
α-Copaene	1371	2.0	0.7	1.8	4.4	4.7	0.6	0.2
β-bourbonene	1380	0.9	–	1.8	0.8	3.4		–
β-Cubebene	1384	1.4	–	1.5	1.1	2.8	–	–
β-Elemene	1387	–	–	–	1.5	–	–	–
α-Gurjunene	1404	–	–	–	1.2	–	–	–
cis-α-Bergamotene	1410	–	–	–	0.9	–		–
α-Caryophyllene	1417	40.6	7.8	23.9	8.0	29.2	7.5	2.5
β-Gurjunene	1427	–	–	0.4	–	–	–	–
trans-α-Bergamotene	1432	–	–	0.9	4.2	–	–	–
Aromadendrene	1435	–	–	–	0.7	–	–	–
(Z)-β-Farnesene	1438	–	–	0.3	–	–	–	–
α-Humulene	1447	1.7	1.1	3.2	2.5	5.4	0.9	0.1
(E)-β-Farnesene	1453	–	–	0.8	1.4	–	–	–
Germacrene D	1475	10.5	3.2	6.6	7.9	8.4	2.8	0.5
β-Selinene	1480	–	–	–	1.5	–	–	–
2-Tridecanone	1489	11.1	36.0	20.4	30.1	2.4	64.1	88.1
β-Bisabolene	1504	–	–	0.8	2.4	–	–	–
β-Sesquiphellandrene	1520	0.5	1.1	3.4	2.6	2.4	0.6	–
(E)-Nerolidol	1560	–	–	0.4	1.2	–	–	–
Spathulenol	1571	–	1.6	2.7	0.8	1.1	–	0.1
Caryophyllene oxide	1577	2.7	6.9	4.4	5.6	15.4	2.8	0.3
2-Tetradecanone	1584	–	–	–	1.2	1.5	0.8	0.1
Carotol	1589	–	–	0.6	2.2	–	–	–
Humulene epoxide II	1601	–	1.4	0.3	–	1.7	–	–
Unidentified hydrocarbon	1645	0.5	6.9	3.1	–	0.8	0.5	–
2-Pentadecanone	1650	28.1	26.4	20.8	6.7	14.2	18.9	6.9
Unidentified sesquiterpenes		–	4.1	–	6.6	5.4	–	1.2
% Total		100.0	97.2	98.4	98.3	98.8	99.5	100.0

*RI = Retention Index, on SE-54 and DB-5ms columns.

D, os valores mais altos foram, também, nos óleos das folhas. Em geral, os óleos das folhas se apresentaram muito distintos em relação aos galhos finos, assim como também no mesmo espécime.

Palavras-chave: *Pilocarpus microphyllus*, Rutaceae, jaborandi, composição do óleo essencial, 2-tridecanona, 2-pentadecanona, sesquiterpenos.

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