

ESSENTIAL OILS FROM FOUR *PIPER* SPECIESA. P. MARTINS, L. SALGUEIRO,* R. VILA,† F. TOMI,‡ S. CAÑIGUERAL,† J. CASANOVA,‡
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Abstract—The essential oils from *Piper capense*, *P. nigrum*, *P. guineense* and *P. umbellatum* from S. Tomé e Príncipe were investigated for the first time. They were analysed by GC, GC-mass spectrometry and ¹³C NMR. Monoterpene hydrocarbons were the main group of constituents in three of the samples (*P. capense*, *P. nigrum* and *P. umbellatum*), whereas for the other species (*P. guineense*) phenylpropanoid derivatives were the most important ones. β -Pinene (32.5%) and β -caryophyllene (12.6%) were the major compounds in the volatile oil of *P. capense*. Dillapiole (44.8%), followed by myristicin (9.8%), were the main constituents of *P. guineense*. The most important constituents in the essential oil of *P. nigrum* were limonene (18.8%), β -caryophyllene (15.4%), sabinene (16.5%) and β -pinene (10.7%). The essential oil of *P. umbellatum* was characterised by its high β -pinene (26.8%), α -pinene (17.6%) and (E)-nerolidol (12.4%) content.
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INTRODUCTION

The Republic of S. Tomé e Príncipe, located in West Africa's Gulf of Guinea some 180 miles off the coast of Gabon, is one of the smallest states in the world. In S. Tomé, like in many other developing countries, there is a treasure of traditional medicine and traditions concerning naturally occurring drugs, based on the empirical knowledge of medicinal and toxic plants, gained by the ancestors and passed on from generation to generation by oral tradition. During an ethnobotanical survey, it was verified that essential oil-bearing plants are widely used in this country. They are utilised in different forms, such as whole herbs, powders, extracts and vapours, for a variety of purposes.

As part of an exhaustive research of the composition of the essential oils of the aromatic and medicinal plants from S. Tomé e Príncipe, we report herein the results of the investigation of four *Piper* species widely used by local traditional healers, *P. capense*, *P. guineense*, *P. nigrum* and *P. umbellatum*, for which no data have been previously reported.

The leaves of *P. capense*, known in S. Tomé as “Fiá Boba Piquina”, are used as a stomachic and carminative in indigestion, flatulence and colic. It is said to cause sweating and sleepiness. The leaves of *P. guineense*, whose vernacular name is “Pó Pimenta” and, in other countries, known as “Ashanti pepper”, are widely used as an antibacterial, especially to heal wounds. Its pulverised seeds are used as insecticide. The fruits of *P. nigrum*, whose vernacular name is “Pimenta”, are used as a condiment and when applied externally, as a stimulant, rubefacient and disinfectant. The leaves of *Piper umbellatum*, known as “Fiá Boba d'Obô”, are used to heal wounds and to reduce swellings and skin irritations.

The essential oils of these species were obtained by hydrodistillation of the aerial parts (*P. capense*, *P. guineense* and *P. umbellatum*) and fruits (*P. nigrum*). Qualitative and quantitative analysis of the oils were performed by GC and GC-mass spectrometry, using two fused silica capillary columns of different stationary phases, and by ¹³C NMR. The latter has proved to be a useful technique in the investigation of the composition of essential oils [1]. On the basis of previous work [2], we have developed a technique which allows the identifi-

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Table 1. Constituents of essential oils from *Piper* species from S. Tomé e Príncipe

Components*	% in samples			
	<i>P. cap</i>	<i>P. guin</i>	<i>P. nig</i>	<i>P. umb</i>
α -Thujene	0.1	0.1	1.4	0.2
α -Pinene	8.6	1.1	5.7	17.6
Camphene	0.2	0.7	0.1	0.8
6-Methyl-5-hepten-2-one	—	—	—	0.1
β -Pinene	32.5	0.3	10.7	26.8
Sabinene	0.7	0.1	16.5	—
Myrcene	1.0	0.3	2.0	0.2
α -Phellandrene	1.8	8.2	0.7	0.1
α -Terpinene	—	—	0.2	0.2
δ -3-Carene	0.3	—	1.7	—
<i>p</i> -Cymene	0.3	0.9	0.2	0.3
(<i>Z</i>)- β -Ocimene	0.2	0.1	—	6.8
Limonene	3.6	2.6	18.8	3.8
β -Phellandrene	2.2	5.9	2.9	0.6
(<i>E</i>)- β -Ocimene	—	0.1	0.5	1.4
γ -Terpinene	—	0.1	0.4	—
<i>trans</i> -Sabinene hydrate	—	—	0.3	0.1
2-Nonanone	—	—	—	0.2
Terpinolene	0.2	—	0.4	0.4
<i>p</i> -Cymenene	—	—	—	0.2
Linalool	3.8	3.4	1.1	7.9
<i>cis</i> -Sabinene hydrate	0.3	0.1	0.1	0.2
1,3,8- <i>p</i> -Menthatriene	—	—	—	0.2
Camphor	—	0.8	—	—
Isopulegol	0.4	—	—	—
Borneol	—	—	—	0.6
Terpinen-4-ol	—	—	1.7	0.8
α -Terpineol	—	—	—	0.5
δ -Terpineol	—	—	0.5	—
Piperitol†	0.3	—	—	—
Piperitone	0.6	1.2	—	—
Benzenepropanoic acid, methyl ester	0.2	—	—	—
Bornyl acetate	—	—	—	0.1
Thymol	—	—	—	0.1
Benzenepropanoic acid, ethyl ester	7.8	—	—	—
δ -Elemene	0.8	0.1	1.9	1.0
α -Cubebene	0.2	0.1	—	—
Methyl eugenol	0.2	—	—	—
α -Copaene	1.2	1.9	1.2	—
β -Elemene	—	1.1	0.8	—
α -Cedrene	—	—	0.3	—
β -Caryophyllene	12.6	1.2	15.1	9.8
α -Gurjunene	0.4	0.1	0.2	—
<i>E</i> - β -Farnesene	—	0.1	—	0.4
α -Humulene	1.1	0.5	1.1	0.7
<i>allo</i> -Aromadendrene	0.2	0.1	—	0.2
Germacrene D	3.3	1.5	—	—
γ -Muurolene	2.1	—	0.5	0.1
β -Selinene	—	0.5	0.7	—
Myristicin	0.2	9.8	—	—
Ledene	0.4	1.0	—	—
Zingiberene	—	—	0.2	—
Furanodiene	—	—	2.1	—
(<i>E,E</i>)- α -Farnesene	—	—	—	0.7
α -Selinene	—	0.8	0.6	—
δ -Guaiene	—	0.4	—	—
β -Bisabolene	—	—	1.1	—
β -Curcumene	—	—	0.1	—
Bicyclogermacrene	—	0.6	—	—
δ -Cadinene	1.8	1.6	0.7	0.1
(<i>Z</i>)-Nerolidol	—	—	—	0.4
Elemicin	—	3.4	—	—
β -Elemol	0.2	—	0.3	—
Germacrene B	—	—	1.4	—
β -Caryophyllene oxide	—	—	0.9	—
(<i>E</i>)-Nerolidol	3.2	0.4	0.3	12.4
Germacrene D-4-ol	0.7	0.2	—	—
(<i>E</i>)-2-Tridecenal	0.4	—	—	0.4
Neryl-isovalerate	—	—	—	0.1
Cubenol	0.5	0.2	—	—
T-Cadinol	0.3	—	0.3	0.1
T-Muurolol	0.4	—	0.2	0.2
α -Eudesmol	—	—	1.2	—

Table 1 (continued)

Components*	% in samples			
	<i>P. cap</i>	<i>P. guin</i>	<i>P. nig</i>	<i>P. umb</i>
α -Cadinol	0.6	0.2	—	—
α -Elemol	—	1.0	—	—
Dillapiole	—	44.8	—	—
Apiole	—	0.1	—	—
2 <i>Z</i> ,6 <i>Z</i> -Farnesol	0.2	—	—	0.1
2 <i>E</i> ,6 <i>Z</i> -Farnesol	—	—	—	0.1
2 <i>E</i> ,6 <i>E</i> -Farnesol	—	—	—	0.1
Phytol	0.4	0.2	—	1.0
Monoterpene hydrocarbons	51.7	20.5	62.2	59.6
Oxygenated monoterpenes	5.4	5.5	3.7	10.3
Sesquiterpene hydrocarbons	24.1	11.6	26.2	13.0
Oxygenated sesquiterpenes	6.1	2.1	3.2	13.5
Others	9.2	58.3	2.1	1.7
Total identified	96.4	97.9	97.4	98.2

*Components are listed in order of elution on methylsilicone SE 30 column.

†Specific isomer not determined.

P. cap: *Piper capense*; *P. guin*: *Piper guineense*; *P. nig*: *Piper nigrum*; *P. umb*: *Piper umbellatum*.

cation of the main compounds of an oil (detection limits: 0.5–1%) by ^{13}C NMR, without previous fractionation. Identification is performed by computer-aided analysis of the ^{13}C NMR spectrum of the total oil, by comparing the signals obtained with those of pure compounds included in a library created in our laboratory [1]. Each compound is unambiguously identified taking into account the number of identified carbons, the number of overlapped signals and the difference of chemical shift of each resonance in the mixture spectrum and in the reference.

RESULTS AND DISCUSSION

The essential oils were obtained by hydrodistillation of the aerial parts (*P. capense*, *P. guineense* and *P. umbellatum*) and fruits (*P. nigrum*). Yields were, respectively, 0.15, 0.10, 0.13 and 0.26%.

Results obtained for the qualitative and quantitative analysis of the essential oils are shown in Table 1. In total, 81 compounds were identified, accounting for 96.4 to 98.2% of the constituents. Three of the oils were characterised by high percentages of monoterpene hydrocarbons, but they shaved some differences in their main constituents. β -pinene (32.5%) and β -caryophyllene (12.6%) were the major compounds in the volatile oil of *P. capense*. The most important constituents in the oil of *P. nigrum* were limonene (18.8%), β -caryophyllene (15.4%), sabinene (16.5%) and β -pinene (10.7%). The oil of *P. umbellatum* was characterised by its high β -pinene (26.8%), α -pinene (17.6%) and (*E*)-nerolidol (12.4%) content. On the other hand, phenylpropanoid derivatives were the most important group of components in the oil of *P. guineense*,

dillapiole (44.8%) being the main constituent, followed by myristicin (9.8%).

To our knowledge, this is the first time that a detailed composition of the essential oil of *P. capense* is given. A literature search and the available databases showed that the only sesquiterpene mentioned in this species, collected in Zimbabwe, is capentin, and was found in the root; this compound is not present in our sample. This is also the first time that a detailed composition of the oil of *P. umbellatum* is presented. In fact, we have found references to the leaf essential oil of this plant, collected in Brazil, but the only compounds mentioned were cadinene, caryophyllene and phellandrene [3] in unknown quantities. Despite the well known composition of the fruits of *P. guineense* [4], to our knowledge this is also the first time that the composition of the oil from the leaves of this plant is given. Most of the compounds that we have found in the leaf oil are also present in the fruit oil. The major differences found are the percentage of dillapiole (44.8% in the leaf and 0.09% in the fruit) and the presence of safrole (4.8%) and sarisan (15.7%) in the fruit, compounds that we have not found in the leaf oil. The oil of *P. nigrum* fruits is well characterised and the results that we obtained are in accordance with those described previously in the literature for plants of different origins [5–7]. For example, Gopalakrishnan [7] gave the most relevant compounds of three samples that he had analysed, as α -pinene (5.07–6.18%), sabinene (8.50–17.6%), β -pinene (9.16–11.08%), myrcene (2.20–2.30%), limonene (21.06–22.17%), *p*-cymene (0.0–0.18%), β -caryophyllene (21.59–27.70), and oxygenated constituents (3.39–5.68%). The composition of our oil is very close to these, our values being, respectively, 5.7, 16.5, 10.7, 2.0, 18.8, 0.2, 15.1 and 6.9%. Furthermore, we have found, in the oil of *P. nigrum*

fruits some constituents that have not been previously quoted in the literature for this specific essential oil, for instance, furanodiene (2.1%). This compound was identified from the ^{13}C NMR spectrum of the total essential oil and from its mass spectrum which was compared with literature data [8, 9].

Computer-aided analysis of essential oils using ^{13}C NMR spectroscopy has been shown to be a fast and accurate technique which can be used together with GC-mass spectrometry for unambiguous identification of some components of oils [10, 11]. In this way, benzenepropanoic acid ethyl ester and germacrene D-4-ol, constituents found in the oil of *P. capense*, were also identified [12].

EXPERIMENTAL

Plant material

Aerial parts of *P. capense* L., *P. guineense* Schum. et. Thom. and *P. umbellatum* L. and fruits of *P. nigrum* L. were collected in cascata Apaga-Foguinho, S. Manuel, Lagoa Amélia and Diogo Vaz, in May 1995, respectively. Species were identified and authenticated by a plant taxonomist. Voucher specimens are deposited at the Herbarium of the Instituto Botânico de Coimbra (COI) under the numbers MM 489, MM 463, MM 479 and MM 490.

Information on the medicinal uses of the species examined is based on first hand information collected by personal contact with traditional healers, usually villagers, who had knowledge of the curative property of plants. In this study, we aimed at obtaining specific information about which plants and plant parts are used, how the plant material is collected and processed to remedies and finally how the remedies are administered.

Analysis of essential oils

Essential oil contents of air-dried plant material were determined according to Ref. [13]. Analysis of volatile oils obtained by hydrodistillation was carried out by GC and GC-MS using fused silica capillary columns with two different stationary phases (Carbowax 20M and methylsilicone SE-30), as previously reported [10, 11]. Constituents of essential oils were identified on the basis of their GC retention indices (RI) with reference to a homologous series of fatty acid Me esters, and by matching their 70 eV mass spectra with our data and with reference libraries [8, 14]. Most of the compounds with percentages $\geq 1.0\%$ were also identified by ^{13}C NMR [1]. ^{13}C NMR spectra were recorded on a Bruker AC 200 Fourier Transform Spectrometer, operating at 50 MHz, equipped with a 5 mm probe,

in CDCl_3 , with all shifts referred to int. TMS, with the following parameters: pulse width (PW) 3.2 μs , acquisition time 1.3 s for 32 K data table with spectral width (SW) of 250 ppm. The number of accumulated scans was 10 000 for each sample of oil (70 mg in 0.5 ml CDCl_3). ^{13}C NMR were recorded with CDP mode-decoupling and a digital resolution of 0.763 Hz pt^{-1} . Exponential multiplication of the free induction decay with a line broadening of 1 Hz was used before Fourier transformation.

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