

Article

Essential Oil in the Taxonomy of *Ocimum selloi* Benth[#]

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Received: December 9 1995; August 12, 1996

A composição química do óleo essencial das folhas e flores de dois acessos de *Ocimum selloi* Benth., cultivados na Universidade Federal de Viçosa foi analisada. Para o acesso A o componente principal foi identificado como estragol, o qual representa 94,95% e 92,54% do óleo das folhas e das flores, respectivamente. O óleo obtido das folhas e flores do acesso B é constituído de 65,49% e 66,18% de metil eugenol, respectivamente. Para ambos os acessos, diversos constituintes químicos, presentes em quantidades menores, foram identificados. As diferenças fenotípicas e químicas observadas entre os dois acessos estudados indicam a existência de duas variedades quimicamente distintas de *Ocimum selloi* Benth.

Chemical composition analysis of the essential oil from the leaves and flowers of two accessions of *Ocimum selloi* Benth, cultivated at the Federal University of Viçosa was carried out. For accession A the major component was identified as estragole and represented 94,95% and 92,54% of the oil from the leaves and flowers respectively. For accession B, the oil from the leaves and flowers was constituted by 65,49% and 66,18% of methyleugenol, respectively. For both accessions several minor constituents were also identified. The phenotypic and chemical differences observed between these two accessions suggest the existence of two chemically distinct varieties for *Ocimum selloi* Benth.

Keywords: *Ocimum selloi*, *estragole*, *methyleugenol*

Introduction

The genus *Ocimum* (Labiatae) comprises 160 species and is found throughout the tropical and sub-tropical regions of the world¹. The larger genetic diversity of this genus is found in Brazil¹. Several species of this genus has commercial utility as a source of essential oil for the pharmaceutical, food, flavour and perfumary industries².

The specie *Ocimum selloi* Benth known as "alfavaquinha", "anis" and "elixir-paregorico", is originated from South America, and is used in the traditional medicine as antiinflammatory, analgesic and antiespasmodic³.

In the germplasm collection kept at the Federal University of Viçosa, accessions of this specie were found to have different morphological characteristics. The accession having more vigorous plants, with pink corollas and leaves with an anis-like odour was called "A". The accession formed by smaller plants, with darker corollas, reddish calyx and leaves without anis-like odour was called "B". Thus a chemical analysis of the essential oil of these two accessions was carried out in order to see if the phenotypic variability observed was also present at molecular level. Information about the chemical composition of the oil could then be useful in the classification of these plants.

[#] Taken from the MSc dissertation presented by E.R.M. at the Federal University of Viçosa on February 1996.

Material and Methods

Plant material

Seeds of *Ocimum selloi*, accessions A and B, from the germplasm collection of the "Grupo Entre-Folhas-Viçosa (MG)", were cultivated at the Federal University of Viçosa, beginning November 1994 and harvested while in full bloom in April 1995. The aerial parts were collected and the leaves and flowers were separated.

Essential oil extraction

Steam distillation was carried out by passing steam in a 2 L round-bottomed flask containing the fresh plant material (100 g of leaves or 80 g of flowers). A total of 850 mL of distillate was collected, and the oil was extracted with diethyl ether (3 x 80 mL). The combined organic extract was dried over MgSO₄ and concentrated under reduced pressure in a rotary evaporator at 30 °C, and the essential oil obtained was weighted. The extractions were carried out in triplicates, and for each extraction the material (leaves and flowers) were obtained from one single specimen.

Essential oil analysis

The major constituent of the essential oil from each plant group was purified by column chromatography on silica gel, using hexane or hexane:diethyl ether (8:1) as a solvent for groups A and B respectively. The compounds isolated were identified by means of their MS, IR and ¹H-NMR data.

IR spectra were recorded with a Perkin Elmer 599B double beam grating spectrophotometer; ¹H-NMR spectra were recorded with a Bruker WH300 (300 MHz) instrument, using tetramethylsilane as internal standard.

Data for estragole

IR (thin film) $\bar{\nu}/\text{cm}^{-1}$: 3085, 3010, 2990, 2950, 2900, 2830, 1635, 1608, 1580 and 1510; ¹H-NMR (300 MHz, CDCl₃) δ : 3.35 (d, J= 6.5 Hz, CH₂), 3.78 (s, OCH₃), 5.00-5.15 (m, =CH₂), 5.85-6.15 (m, =CH), 6.82 (d, J= 9.0 Hz, Ha/Ha'), 7.10 (d, J= 9.0 Hz, Hb/Hb'); MS m/z (%): 148 (M⁺, 100), 133 (22), 121 (35), 117 (41), 105 (23), 91 (32) and 77 (38).

Data for methyleugenol

IR (thin film) $\bar{\nu}/\text{cm}^{-1}$: 3060, 2990, 2930, 2820, 1635, 1590 and 1510; ¹H-NMR (300 MHz, CDCl₃) δ : 3.35 (d, J= 6.5 Hz, CH₂), 3.85 and 3.87 (2s, 2x OCH₃), 5.02-5.15 (m, =CH₂), 5.85-6.07 (m, =CH), 6.70-6.85 (m, 3H aromatic ring); MS m/z (%): 178 (M⁺, 100), 163 (35), 147 (33), 135 (10), 107 (30), 91 (28) and 77 (14).

Minor constituents were identified by combined GC-MS analysis. These were performed by a model 5890 A GC

system with mass-selective detector Model 5970 (Hewlett-Packard). GC conditions were: direct injection of 1.0 μL sample diluted 10:1 with CHCl₃; fused silica column HP-1 (30 m x 0.25 mm x 0.33 μm); helium as a carrier gas (1.0 mL/min); oven program 80 °C (5 °C/min, 1 min hold) to 250 °C, injector and detector temperature of 150 °C and 225 °C respectively. All spectra were recorded in the electron impact ionization mode at 70 eV. The comparisons of the acquired spectra were made with those found in a Wiley library by an integrated program.

A quantitative analysis of the major chemical constituents of the essential oil was carried out using a Shimadzu GC-14A instrument, equipped with a FID detector, a Carbowax 20M capillary column of 30 m, i.d. 0.25 mm and the following temperature program: 60 °C (1 min) rising to 160 °C at the rate of 5 °C; injector and detector temperatures of 150 °C and 220 °C respectively. Carrier gas used was hydrogen at 1.0 mL/min. Estragole and methyleugenol, purified previously, were used as standards, and the analysis were carried out in triplicates.

Results and Discussion

The essential oil content in the fresh leaves and flowers of accession A were $0.311 \pm 0.043\%$ (average \pm standard deviation) and $0.474 \pm 0.036\%$, respectively, and for accession B $0.210 \pm 0.022\%$ and $0.398 \pm 0.015\%$.

Chromatograms of the essential oils from the leaves and flowers of both accessions of *Ocimum selloi* are shown in

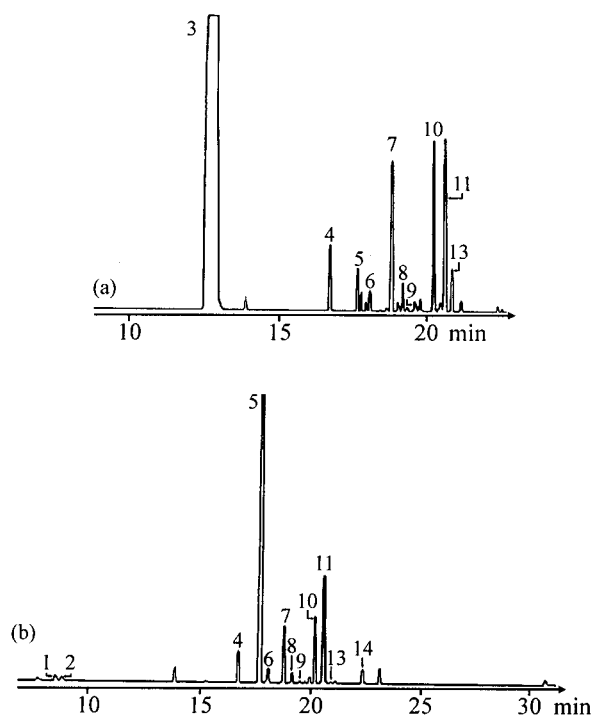


Figure 1. Chromatogram of the essential oil from the leaves of *Ocimum selloi* Benth.: (a) accession A and (b) accession B.

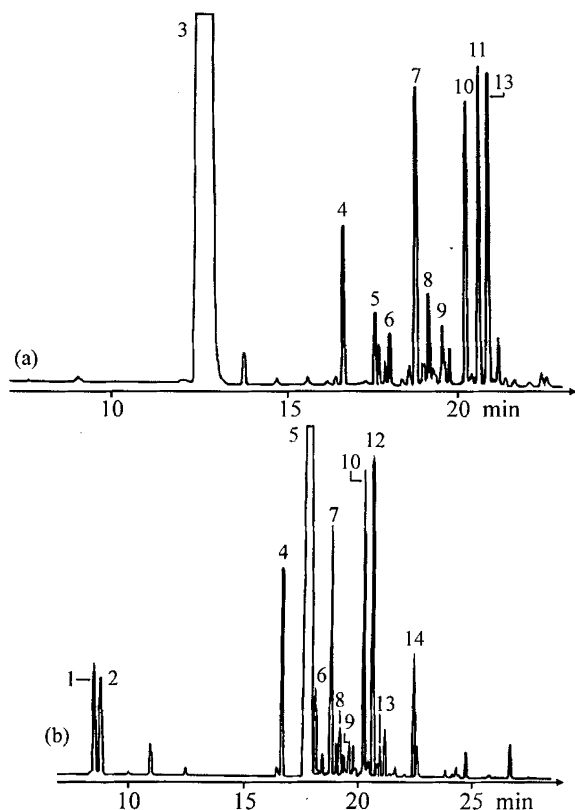


Figure 2. Chromatogram of the essential oil from the flowers of *Ocimum selloi* Benth.: (a) accession A and (b) accession B.

Figs. 1 and 2. The compounds identified are listed in Table 1, and the relative area (%) of each compound is also presented.

The major compound found in the oil from the leaves and flowers of accession A was identified by GC/MS as estragole (Fig. 3) This oil was submitted to fractioning in a column chromatography on silica gel and the major compound was obtained in a pure form. The IR spectrum of this compound was identical to the corresponding data for estragole reported in the literature⁴. The ¹H-NMR spectrum confirmed the proposed structure.

The structure for some of the minor constituents were proposed by comparison of their mass spectrum with the mass spectrometer data bank (Table 1). All these compounds have already been identified from several other species in this genus⁵. Although the lack of pure references samples makes it difficult to have complete confidence in the proposed identification, this is not a problem for the present investigation, as for the differentiation between two chemotypes only the major oil components are considered⁶.

The essential oil of accession B was also submitted to a column chromatography purification and the major constituent was isolated. The structure for this compound was initially proposed from GC/MS data as methyleugenol (Fig. 3) and was further confirmed by analysis of its IR and ¹H-NMR data.

Table 1. Composition of the essential oil from the leaves and flowers of *Ocimum selloi* Benth.

Peak N°.	Component	M.W.	R.T./min	Accession A		Accession B	
				Flower	Leaves	Flower	Leaves
				Area (%)		Area (%)	
1	E or Z ocimene	136	8.53	-	-	2.50	t
2	E or Z ocimene	136	8.82	-	-	2.20	t
3	Estragole	148	12.83	81.81	80.70	-	-
4	?	204	16.70	1.69	1.81	4.60	3.19
5	Methyleugenol	178	17.64	0.79	1.13	63.00	63.08
6	?	204	18.15	0.63	0.56	2.00	1.36
7	<i>Trans</i> -caryophyllene	204	18.83	3.17	4.30	5.50	6.84
8	Bergamotene	204	19.20	0.95	0.79	1.09	0.91
9	α -Humulene	204	19.57	0.37	t*	1.20	t
10	Bicyclosesquiphellandrene	204	20.22	2.96	4.56	6.79	7.76
11	Germacrene B	204	20.62	3.38	4.56	-	11.87
12	Bisabolene epoxide	220	20.67	-	-	7.11	-
13	β -Bisabolene	204	20.87	3.22	1.13	t	2.73
14	?	222	22.40	-	-	2.79	1.37

? sesquiterpene not identified; * t, trace amount.

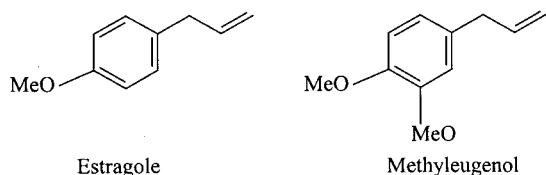


Figure 3. Major constituents from the essential oil of *Ocimum selloi* Benth.

The characterization of the minor constituents was also carried out by GC/MS as previously mentioned (Table 1).

With pure samples of estragole and methyleugenol available to be used as standards, a quantitative analysis of these two components in the oil from the leaves and flowers was carried out by gas chromatography, using the calibration curve method⁷. The percentage of estragole in the oil from the leaves and flowers of accession A was $94.95 \pm 1.02\%$ (average \pm standard deviation) and $92.54 \pm 0.78\%$ respectively. For accession B the percentage of methyleugenol in the oil from the leaves and flowers was $65.49 \pm 3.43\%$ and $66.18 \pm 1.97\%$ respectively.

For each accession the chemical composition of the oil from the leaves and flowers were very similar. For accession B, the major difference observed was the presence of a reasonable amount of compounds **1**, **2** and **9** in the flowers, while in the leaves these compounds were present in trace amounts. Also compound **12** was not present in the leaves and was the second major constituent in the oil from the flowers. On the other hand compound **11** was the second major constituent in the leaves and was not present in the flowers.

For accession A the chemical composition of the oil from the leaves and flowers were very similar in qualitative terms (Table 1).

Between the two accessions, the major difference in the oil chemical composition was the presence of estragole as the major constituent in the leaves and flowers of accession A and its absence in accession B. On the other hand, the major compound found in the oil from the leaves and flowers of accession B was methyleugenol. This compound was found in very small amount in the oil of plants from accession A.

The chemical composition of the essential oil for different *Ocimum* species has been shown to vary greatly. For example, camphor amounts to 70% of the essential oil of *O. kilimadjaricum*⁸ and 65% in the case of *O. canun*⁵. The chemical composition of volatile oil from *O. basilicum* cultivated in different parts of the world has been shown to vary enormously^{5,9}.

Ribeiro *et al.*¹⁰ has shown the existence of two chemotypes of *O. nudicaule* Benth. The one classified as the *anisifolia* variety has estragole as the major constituent of its essential oil, the other that has no estragole was classified as the *nudicaule* variety.

Also the specie *Ocimum basilicum* known as Sweet basil or French basil has two popularly recognized races. One is rich in eugenol and the other is rich in estragole¹¹. Thus, the difference in chemical composition found for the two accessions studied is not unique.

Considering that the plants investigated in this work were cultivated under the same conditions, the morphological and chemical differences observed could not be due to ecological factors, but should be a consequence of genetical difference between the two groups of plants¹⁰. These results suggest that the two accessions studied should constitute two taxonomic varieties of *Ocimum selloi*. A genetic investigation of these two accessions is underway at the moment in order to corroborate, or not, this hypothesis.

Acknowledgments

We thank the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) and the "Cordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) for research (VWDC and LCAB) and graduate (ERM) fellowships.

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