

# Yield and Phytochemical Characterization of Essential Oil from *Ocimum selloi* B. Obtained by Hydrodistillation and Supercritical Fluid Extraction

L. Aparecida Salgado de Moraes<sup>1,a</sup>, Lin Chau Ming<sup>2</sup>, M. Ortiz Mayo Marques<sup>3</sup> and M.Á. Almeida Meireles<sup>4</sup>

<sup>1</sup> Embrapa Meio Ambiente; Laboratório de Produtos Naturais, Rod. SP 340, Km 127,5, Bairro Tanquinho Velho, Jaguariúna, SP, CEP 13820-000, Brazil

<sup>2</sup> Faculdade de Ciências Agronômicas, UNESP, Campus Fazenda Experimental Lageado, Departamento de Horticultura, Caixa Postal 237 - CEP 18610-307, Botucatu, SP, Brazil

<sup>3</sup> Centro de Pesquisa e Desenvolvimento de Recursos Genéticos Vegetais, Caixa Postal 28, CEP 13001-970 Campinas, SP, Brazil

<sup>4</sup> Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Departamento de Engenharia de Alimentos, Caixa-Postal: 6121, CEP 13083-862, Campinas, SP, Brazil

**Keywords:** methyl-chavicol; trans-anethole; SFE; organic treatment; mineral treatment.

## Abstract

The yield and chemical composition of essential oils from leaves of *Ocimum selloi* B. submitted to organic and mineral fertilization, obtained by hydrodistillation and supercritical fluid extraction (SFE) were compared. Essential oil was extracted in a Clevenger-type apparatus for 2 h 30 min and analyzed by GC-MS (Shimadzu, QP 5050-DB-5 capillary column - 30 m × 0.25 mm × 0.25 μm). Carrier gas was helium (1.7 ml/min); split ratio: 1:30. Temperature program: 50°C, rising to 180°C at 5°C/min, 180°C, rising to 280°C at 10°C/min. Injector temperature: 240°C and detector temperature: 230°C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known compounds reported in the literature. An Applied Separations-apparatus (Speed SFE, model 7071, Allentown, PA, EUA) was used for SFE extractions. They were conducted at pressure 200 bar and temperature 30°C (20 min in static mode and 40 min in dynamic mode). The supercritical CO<sub>2</sub> flow rate was (6.8±0.7)×10<sup>-5</sup> kg-CO<sub>2</sub>/s. The essential oil collected was immersed in ethylene glycol bath (5°C). The yield of essential oils obtained by SFE was larger than hydrodistillation in both fertilization treatments (279 and 333% for organic and mineral fertilizations, respectively). There were no differences between the fertilization treatments. The amount of the volatile components showed by GC-MS chromatogram was highest in the essential oil obtained by hydrodistillation than SFE. The main volatile constituents of the essential oils were trans-anethole (Hydrodistillation: organic - 52.4%; mineral - 55.0%/ SFE: Hydrodistillation - 62.8%; mineral - 66.8%) and methyl-chavicol (Hydrodistillation: organic - 37.3%; mineral - 38.3%/ SFE: organic - 8.4%; mineral - 4.3%). A reduction of methyl-chavicol relative proportion of essential oil obtained by SFE was observed. Cys-anethole, α-copaene, trans-cariofilene, germacrene-D, β-selinene, biclogermacrene and spathulenol were expressed only in hydrodistillation. The extraction of essential oil by SFE presented larger yield of essential oil than hydrodistillation technique, presenting, however, these essential oils, different phytochemical profiles.

## INTRODUCTION

*Ocimum selloi* B. is a native species from Brazil that produces essential oil. It presents potential use in popular medicine and it is known as “elixir paregórico” in Rio de Janeiro and Espírito Santo States, as “anis and alfavaquinha” in Minas Gerais State and “atroveran” in São Paulo State, Brazil. These popular terms are used because their chemical and pharmacological properties, and its similarity to other species of the

<sup>a</sup> lilia@cnpma.embrapa.br

*Ocimum* genus. It has been used as anti-diarrhea, anti-spasmodic and anti-inflammatory. These properties were observed in pre-clinic assays (Vanderlinde et al., 1994).

The extraction of essential oils by supercritical fluid extraction has been used as an alternative technique to conventional process, because this methodology reduces the problems with decomposition, due the utilization of lower extraction temperatures; it produces organic solvents residues free extracts and it permits to increase the yield of extraction and to obtain total extracts or enriched fractions of active principle, by changing operational conditions (pressure, temperature and others).

In despite of the potential use of *Ocimum selloi* as flavorizant and mainly as a medicinal plant, there are few studies about this species, under phytochemical and agronomic aspects. So, studies about techniques of cultivation of *O. selloi* on aerial phytomass production, yield and chemical composition of essential oil can contribute to establish agronomical parameters of this species.

Research about supercritical fluid extraction of essential oil will furnish relevant information that can contribute to increase the yield of essential oil or its main compounds. It could increase the value of raw material.

The aim of this work was to compare the yield and chemical composition of essential oils from leaves of *Ocimum selloi* B. submitted to organic and mineral fertilization, obtained by hydrodistillation and supercritical fluid extraction (SFE).

## MATERIAL AND METHODS

The experiment of growth in the field was conducted at the Experimental Farm Lageado/Faculdade de Ciências Agrônômicas (FCA), UNESP, Botucatu District, SP-Brazil. Drying of raw material and extraction of essential oils by hydrodistillation was developed in the Department of Plant Production, Horticulture, FCA, UNESP. Analyses of chemical composition of essential oils were carried out at the Natural Products Laboratory of Center for R&D Plant Genetic Resources of Instituto Agrônômico de Campinas (IAC) and the extraction of essential oils by supercritical fluid extraction (SFE) was accomplished on LASEFI (Laboratory of Supercritical Technology: Extraction, Fractionation, and Identification of vegetable extracts) that belongs to the Department of Food Engineering (DEA), College of Food Engineering (FEA), State University of Campinas (UNICAMP), São Paulo State, Brazil.

Plant samples collected were submitted to organic fertilization (cattle manure, 5000 kg/ha) and mineral fertilization (NPK - ammonium sulfate, 75 kg/ha; single superphosphate, 50 kg/ha; potassium chloride, 105 kg/ha). For essential oil extraction, dried leaves (50.0 g) and inflorescences (30.0 g) were submitted to hydrodistillation in a Clevenger-type apparatus for 2 h 30 min. Extraction time was optimized through preliminary tests, observing the volume of essential oil (every 30 min), considering the optimal time, when no more changes were observed in the total volume. Essential oils extracted were transferred to transparent glass vials (5 ml) with stopper and screw cap, pre-weighed on an analytical balance (AND, model HR-120) and kept in a freezer until the moment of chemical composition analysis. Extractions were performed in triplicate. The yield of essential oils was expressed as the average value of three replicates and calculated using the following equation:

$$\% \text{ yield} = [\text{Average mass of essential oil (g)} \times 100] / \text{part of plant mass (g)} \quad (1)$$

For the extraction with supercritical carbon dioxide, dried leaves of *Ocimum selloi* were triturated in a mill (Tecnal mod. TE-631 /1, Brazil) at 20,500 rpm for 15 s. Particle size distribution was determined using sieves of 24, 32, 48, 80 and 100 mesh with the aid of a sieve shaker (Bertel, CATEL, Caieiras, São Paulo State, Brazil). In the extraction experiments, mixture of particles with size distribution from 32 to 80 mesh were used. Essential oil extractions from *O. selloi* were realized on Applied Separations apparatus (Speed SFE, model 7071, Allentown, PA, EUA), in an extraction column with capacity of 5 ml (Thar Designs, CL 1165, Pittsburgh, PA, EUA, with internal volume of 6 ml).

Triturated raw material ( $2.51 \pm 0.01$  g), composed by a mixture of granulometric distribution of 32 mesh ( $26 \pm 2\%$ ), 48 mesh ( $52 \pm 1\%$ ) and 80 mesh ( $21 \pm 2\%$ ) was added in the column extraction and packaged (height: 0.019 m; length: 0.020 m) with the help of a steel shank (diameter: 0.014 m). Extraction column was connected to the equipment. Pressure (200 bar) and temperature ( $30^\circ\text{C}$ ) operating conditions were set, keeping the static period of carbon dioxide ( $\text{CO}_2$ , 99.98% purity, Gama Special Gases, Campinas, São Paulo State, Brazil), in contact with the particles for 5 min. The flow of  $\text{CO}_2$  was  $(6.8 \pm 0.7) \times 5.10$  kg  $\text{CO}_2/\text{s}$ . The essential oil was collected in glass bottle (50 ml) immersed in a bath of ethylene glycol ( $5^\circ\text{C}$ ) in order to reduce losses of more volatile components. Extraction time was about 1 h, approximately. After removal of the column system, cleaning of the line extraction with ethyl acetate (P.A., Lot. 55893, LabSynth, São Paulo, Brazil) was carried out and the concentrated solution, placed on the rotary evaporator (Heildoph Instruments GMBH, Laborota 4001, Schwabach, Germany) with vacuum controller (Heildoph Instruments GMBH, Rotovac/Rotovac Control, Schwabach, Germany) and stored in a glass flask. Yield of essential oil was determined by weighing the storage and cleaning flasks (Sartorius, model A200S,  $\pm 0.0001$  g, Goettingen, Germany), at the beginning and at the end of extraction and after solvent concentration (ethyl acetate), respectively.

The essential oil extracted was analyzed by GC-MS (Shimadzu, QP-5000, with DB - 5 - capillary column:  $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ). Helium was used as the carrier gas at the constant flow of 1.7 ml/min. The samples were injected in split mode (split ratio 1:30) and the column temperature was programmed from the initial temperature of  $50^\circ\text{C}$ , rising to  $280^\circ\text{C}$  ( $50^\circ\text{C}$  (5 min)- $180^\circ\text{C}$  (5 min);  $180$ - $280^\circ\text{C}$  ( $10^\circ\text{C}/\text{min}$ )) (Moraes et al., 2002). The injector temperature was  $240^\circ\text{C}$  and detector temperature was  $230^\circ\text{C}$ . The mass spectrometer was operated in the 70 eV. Essential oils were dissolved in ethyl acetate (5 mg oil/1 ml solvent).

Identifications of chemical compounds were made by matching their mass spectra with the database of the GC-MS (Nist 62 Libr), literature (McLafferty and Stauffer, 1989) and Kovats retention index (Adams, 1995). The data of yield and chemical composition were submitted to ANOVA, followed of Tukey's test.

## RESULTS AND DISCUSSION

The yield of essential oils obtained by supercritical fluid extraction was higher than hydrodistillation (Table 1), corresponding to 279 and 333% for organic and mineral treatments, respectively. Regardless of the treatment used in cultivation (organic and mineral fertilizers) the chemical composition of essential oils of *Ocimum selloi* obtained by two techniques are different (Table 2). Essential oils obtained by hydrodistillation showed high amount of compounds compared to essential oils obtained by supercritical fluid extraction. The major compounds observed for two techniques were trans-anethole (52.4 a 66.8%) and methyl-chavicol (4.3 and 38.3%), with a reduction in the relative proportion of methyl chavicol extracted by supercritical fluid extraction.

Lower number of compounds, mainly the most volatiles, on essential oils obtained by supercritical fluid extraction may be due to loss by drag at the moment of gas decompression. This problem could be minimized by using two alternatives: capture of volatiles in a glass column with adsorbent material, Porapaq for example, after the collector flask of essential oil or change in operating conditions (pressure, temperature and flow of the solvent). In the essential oils extracted supercritical fluid extraction, a heavier fraction is present, and it is composed by higher molecular weight not identified substances (28.8 and 28.9%). This factor (heavy fraction) associated with a high yield of supercritical fluid extraction indicates that this technique may be drawing other classes of substances not belonging to essential oils. Results like these were noticed by Rodrigues et al. (2003). Essential oil of fennel obtained by supercritical fluid extraction showed besides essential oil compounds, including the major component (anethole), fatty acids and hydrocarbons after fractionation of oil-resin by preparative thin layer chromatography.

## CONCLUSION

The results obtained by this assay showed that yield of essential oil obtained by supercritical fluid extraction (SFE) was higher than hydrodistillation, but differences in the chemical characteristics of essential oils were observed.

## Literature Cited

- Adams, R.P. 1995. Identification of essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publ. Corp, Carol Stream.
- McLafferty, F.W. and Stauffer, D. 1989. The Wiley/NBS Registry of Mass Spectral Data, v. 1-6, John Wiley Sons, New York.
- Moraes, L.A.S., Facanali, R., Marques, M.O.M., Ming, L.C. and Meireles, M.A.A. 2002. Phytochemical characterization of essential oil from *Ocimum selloi*. An. Acad. Bras. Ciênc. 74:183-186.
- Rodrigues, V.M., Rosa, P.T.V.E., Marques, M.O.M., Petenate, A.J. and Meireles, M.A.A. 2003. Supercritical extraction of essential oil from aniseed (*Pimpinella anisum* L.) using CO<sub>2</sub>: solubility, kinetics, and composition data. Journal of Agricultural and Food Chemistry. Estados Unidos 51:1518-1523.
- Vanderlinde, F.A., Costa, E.A. and D'angelo, L.C.A. 1994. Atividades farmacológicas gerais e atividade antiespasmódica do extrato etanólico de *Ocimum selloi* Benth. (elixir paregórico). In: Anais do Simpósio de Plantas Mediciniais do Brasil, Fortaleza, UFCE.

## Tables

Table 1. Yield of essential oils of *Ocimum selloi* leaves extracted by hydrodistillation and supercritical fluid extraction (200 bar and 30°C).

Techniques	Yield (%)	
	Organic treatment	Mineral treatment
Hydrodistillation	0.83	0.81
Supercritical fluid extraction	2.32	2.70

Table 2. Chemical composition of essential oils of leaves from *Ocimum selloi* submitted to organic and mineral treatments, extracted by hydrodistillation and supercritical fluid extraction (200 bar and 30°C).

Substances (%)	%			
	Hydrodistillation		Supercritical fluid extraction	
	Organic treatment	Mineral treatment	Organic treatment	Mineral treatment
Methyl chavicol	37.3	38.3	8.4	4.3
Cys-anethole	1.6	1.7	--	--
Trans-anethole	52.4	55.0	62.8	66.8
$\alpha$ -copaene	1.0	0.5	--	--
Trans-cariofilene	1.7	0.9	--	--
Germacrene D	1.1	0.5	--	--
$\beta$ -selinene	1.9	1.1	--	--
Bicyclogermacrene	1.8	0.9	--	--
Spathulenol	tr	0.4	--	--
Cariophyllene oxide	0.4	0.4	--	--
N.I.	0.8	0.3	28.8	28.9

tr = traces <0.20%; NI = not identified.