

Chemical Constituents of Essential Oils Extracted from the Leaves and Flowers of *Spiranthera odoratissima* A. St. Hil. (Rutaceae)

Fernando Duarte Cabral¹, Cassia Cristina Fernandes Alves¹, Rodrigo Sebastião Cruvinel Cabral¹, Guilherme Brinker Willrich², Antônio Eduardo Miller Crotti² and Mayker Lazaro Dantas Miranda^{3*}

¹Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Rio Verde, Rio Verde, GO, Brazil

²Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, SP, Brazil

³Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Campus Uberlândia Centro, Uberlândia, MG, Brazil

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Abstract: *Spiranthera odoratissima* A. St. Hil. (Rutaceae), a plant grown in midwestern Brazil, has been used for treating rheumatism, infection and abdominal pain. This research aims to investigate chemical constituents of essential oils from *S. odoratissima* A. St. Hil. leaves and flowers. Essential oils were obtained by hydrodistillation and fully characterized by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Results of the essential oil from *S. odoratissima* A. St. Hil. leaves showed that it has 28 volatile compounds which represent 93.8% of the oil. Its major compounds were β -caryophyllene (23.8%), bicyclogermacrene (10.8%), δ -cadinene (7.1%), germacrene D (5.9%), α -copaene (5.5%) and β -elemene (5.3%). Results of the essential oil from *S. odoratissima* A. St. Hil. flowers showed that it has 29 volatile compounds which represent 94.4% of the oil. Its main constituents were β -caryophyllene (14.1%), spathulenol (8.1%), γ -cadinene (7.2%), α -cadinol (6.6%), α -copaene (6.4%), τ -muurolol (6.3%), δ -cadinene (5.8%), β -elemene (5.4%) and alloaromadendrene (5.2%). In this research, chemical constituents of the essential oil from *S. odoratissima* A. St. Hil. flowers were investigated for the first time.

Keywords: *Spiranthera odoratissima* A. St. Hil.; Rutaceae; essential oils; β -caryophyllene; medicinal plant.
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* Corresponding author: E-Mail: maykermiranda@iftm.edu.br (Mayker Miranda); Phone: +34-32292538

1. Plant Source

S. odoratissima A. St. Hil. leaves and flowers (S16°24'11.2''S and 51°06'41.4''W) were collected in November 2017 in Iporá, GO, Brazil. The plant was identified by the botanist Erika Amaral, M. Sc., and a voucher specimen (#1039) was deposited in the herbarium in Rio Verde, at the Instituto Federal Goiano (IFGOIANO).

2. Previous Studies

The genus *Spiranthera* (Rutaceae) comprises three species distributed in South America, mainly in Brazil, Bolivia, Guyana and Venezuela [1]. Regarding its chemical composition, the species *S. odoratissima* contains furoquinoline and β -indoloquinazoline alkaloids, coumarin, terpene, steroid and limonoids [2]. *S. odoratissima* A. St. Hillaire, which is a shrub found in the Brazilian Cerrado, has been known in Brazil as manacá [3]. In folk medicine, its leaves and roots have been widely used for treating various diseases, such as syphilis, rheumatism, renal infections, urinary retention, abdominal pain, gout, furuncles and acne [4]. Anti-inflammatory, analgesic, anxiolytic and antiprotozoal activities of essential oils from *S. odoratissima* A. St. Hil. have been reported in the literature [4].

In this research, chemical constituents of essential oils from *S. odoratissima* A. St. Hil. leaves and flowers collected in Iporá, GO, Brazil, were identified. This is the first time that the analysis of chemical constituents of essential oils from *S. odoratissima* A. St. Hil. flowers has been conducted.

3. Present Study

S. odoratissima A. St. Hil. leaves and flowers were subjected to hydrodistillation for 2 hours by a Clevenger-type apparatus. In order to carry out the analysis, 300 g plant material was divided into three 100-g samples and 500 mL distilled water was added to each sample. After manual collection of essential oil samples, traces of remaining water in the oils were removed with anhydrous sodium sulfate, which was followed by filtration. The extraction procedure was done in triplicate. The isolated oil was stored under refrigeration up to the analysis and test. Yields (w/w) were calculated from fresh leaf and flower weight and expressed as the average of the triplicate analyses.

Gas chromatography (GC) analyses were performed by a Shimadzu GC2010 plus gas chromatograph equipped with an AOC-20s autosampler and fitted with flame ionization detector (FID) and a data-handling processor. An Rtx-5 (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30-m x 0.25-mm i.d.; 0.25- μ m film thickness) was employed. Operation conditions were as follows: column temperature programmed to rise from 60 to 240 °C at 3 °C/min and then hold at 240 °C for 5 min; carrier gas = He (99.999 %), at 1.0 mL/min; injection mode; injection volume, 0.1 μ L (split ratio of 1:10); and injector and detector temperatures = 240 and 280 °C, respectively. Relative concentrations of components were obtained by peak area normalization (%). Relative areas were the average of triplicate GC-FID analyses.

GC-MS analyses were carried out by a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column was an RTX-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary one (30 m x 0.25 mm i.d. x 0.25 μ m film thickness). Electron ionization mode occurred at 70 eV. Helium (99.999 %) was employed as the carrier gas at a constant flow of 1.0 mL/min. The injection volume was 0.1 μ L (split ratio of 1:10). Injector and ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as the one used for GC. Mass spectra were taken at a scan interval of 0.5 s, in the mass range from 40 to 600 Da. Identification of volatile components of *S. odoratissima* A. St. Hil. leaves and flowers (Table 1) was based on their retention indices on an RTX-5MS capillary column under the same operating conditions as the ones found in the case of GC, related to a homologous series of *n*-alkanes (C₈-C₂₀). Structures were computer-matched with the Wiley 7, NIST 08 and FFNSC 1.2 spectra libraries and their fragmentation patterns were compared with literature data [5].

Table 1. Chemical constituents of essential oils from *S. odoratissima* A. St. Hil. leaves and flowers

Compounds	RI _{exp}	RI _{lit}	% RA	
			Leaves	Flowers
limonene	1030	1031	0.5	0.5
linalool	1101	1098	-	1.0
terpinen-4-ol	1171	1177	-	0.3
α -terpineol	1190	1192	-	0.4
bicycloelemene	1327	1331	1.3	0.3
α -cubebene	1358	1352	2.3	2.1
α -copaene	1365	1372	5.5	6.4
β -elemene	1389	1392	5.3	5.4
β -caryophyllene	1416	1418	23.8	14.1
α -bergamotene	1430	1436	0.6	1.6
α -humulene	1447	1455	4.8	3.9
alloaromadendrene	1455	1461	2.6	5.2
γ -muurolene	1472	1477	1.1	1.9
germacrene D	1478	1480	5.9	3.3
β -selinene	1482	1485	1.5	0.9
bicyclogermacrene	1490	1496	10.8	4.2
α -muurolene	1494	1499	—	2.7
germacrene A	1501	1503	0.4	0.4
γ -cadinene	1510	1513	3.0	7.2
δ -cadinene	1521	1524	7.1	5.8
α -cadinene	1533	1538	0.3	0.7
germacrene B	1554	1561	0.5	-
spathulenol	1581	1578	2.9	8.1
caryophyllene oxide	1585	1583	1.6	3.0
viridiflorol	1591	1595	1.2	0.7
epiglobulol	1601	1598	-	0.4
carotol	1613	1597	0.3	-
humulane-1.6-dien-3-ol	1618	1619	0.3	-
isoaromadendrene epoxide	1623	1622	0.5	0.4
τ -muurolol	1643	1640	4.3	6.3
δ -cadinol	1647	1645	0.5	0.6
α -cadinol	1658	1653	4.7	6.6
nootkatone	1810	1807	0.2	-
Monoterpene hydrocarbons			0.5	0.5
Oxygenated monoterpenes			-	1.7
Sesquiterpene hydrocarbons			76.6	66.1
Oxygenated sesquiterpenes			16.7	26.1
Total			93.8	94.4

RI_{exp}: Retention index relative to *n*-alkanes (C₈–C₂₀) in the Rtx-5MS column; RI_{lit}: Retention index found in the literature [5]. RA%: relative area.

Twenty-eight components were identified in oils from *S. odoratissima* A. St. Hil. leaves, which exhibited about 93.8% of the total composition of the oil, whereas twenty-nine components were identified in oils from its flowers, which showed about 94.4% of the total composition of the oil. Major constituents of essential oils from its flowers were β -caryophyllene (14.1%), spathulenol (8.1%), γ -cadinene (7.2%), α -cadinol (6.6%), α -copaene (6.4%), τ -muurolol (6.3%), δ -cadinene (5.8%), β -elemene (5.4%) and alloaromadendrene (5.2%). Major constituents of essential oils from its leaves were β -caryophyllene (23.8%), bicyclogermacrene (10.8%), δ -cadinene (7.1%), germacrene D (5.9%), α -copaene (5.5%) and β -elemene (5.3%). The chemical composition of essential oils from *S. odoratissima* A. St. Hil. leaves was similar to the one previously described in the literature [4]. This research on essential oils from *S. odoratissima* A. St. Hil. adds relevant chemotaxonomic information to the literature, since there have been reports of the isolation of furoquinoline and β -indoloquinazoline alkaloids, coumarin, terpene, steroid and limonoids of this species [6]. Chemical constituents of oils from *S. odoratissima* A. St. Hil. are described in Table 1. The second step will be to evaluate biological activities of these essential oils in order to value this species with a special ecological character.

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ORCID

Fernando Duarte Cabral: [0000-0002-5090-5946](https://orcid.org/0000-0002-5090-5946)

Cassia Cristina Fernandes Alves: [0000-0003-2004-3166](https://orcid.org/0000-0003-2004-3166)

Rodrigo Sebastião Cruvinel Cabral: [0000-0001-8436-0529](https://orcid.org/0000-0001-8436-0529)

Guilherme Brinker Willrich: [0000-0002-6820-8356](https://orcid.org/0000-0002-6820-8356)

Antônio Eduardo Miller Crotti: [0000-0001-6047-4871](https://orcid.org/0000-0001-6047-4871)

Mayker Lazaro Dantas Miranda: [0000-0003-4689-572X](https://orcid.org/0000-0003-4689-572X)

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