Chemical characterization of mint (*Mentha* spp.) germplasm at Federal District, Brazil

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ABSTRACT: Chemical characterization of mint (*Mentha* spp.) germplasm at Federal District, Brazil. The main goal of this work was to evaluate and compare Mint genotypes (*Mentha* spp.) at the Brazil Federal District conditions. Twenty-one genotypes introduced from Purdue University, USA collection and one Brazilian genotype were analyzed based on volatile oil content and relative percentage of essential oil constituents. The essential oil was extracted by hydrodistillation in a Clevenger apparatus. The collected and stored oil was analyzed by gas chromatography and gas chromatography connected to mass spectrometry, to characterize the chemical composition of each genotype. Leaves productivity ranged between 575 and 4,271 Kg/ha and essential oil content from 0.47 to 4.17%. The major essentail oil constituents detected were: 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide. Some genotypes were found to have essential oil with a high content of a particular constituent, like piperitone oxide (79.0 % in CM 4 – *M. suavelons*), carvone (72.1 in CM 2; 70.9% in CM 29) and linalool (78.5% in CM 24 – *M. arvensis*). The genotype CM 20 presented the highest content of essential oil (4.17 %) and menthol yield (65%), but presented lower leaves productivity in District Federal region.

Key words: chemical characterization, genetic resources, essential oil, *Mentha* spp.

INTRODUCTION

The genus *Mentha* (Lamiaceae) consists of 19 species distributed in the Old and New World. Mint species are famous all over the world for their essential oils. The aromatic leaves of mint are used fresh and dried as flavorings or spices in a wide variety of foods. They contain biologically active constituents and are also used in traditional ceremonial rituals and as medicines. Volatile oils of mint species are used to flavor foods, in dental and oral products and in fragrances.

Mint volatile oils composition is largely well known in the literature (Clark, 1998; Czepack, 1998; Maia, 1998). The great differences in the essential oil composition found in members of the genus are reflected in the number of commercial constituents obtained (menthol, menthone, carvone, limonene, linalool, menthyl acetate, piperitone, and pulegone).

Menthol is used in confectionery, perfumery and cigarettes. It is also known in mild local anesthetic, antiseptic, internally as a carminative and gastric sedative. Menthone is used as perfume and flavor compositions and carvone as flavor in liqueurs, perfumery and oral hygiene products (Bauer, 1997). Limonene is an antioxidant, and can be used as solvent, wetting and dispersing agent. Linalool is used in perfumery instead of Bergamot or french lavander oil, since it has similar odor. Menthyl acetate is used in perfumery and in toilet waters having a lavander odor. Piperitone is used in masking odors in dentifrices. Pulegone has a pleasant odor, midway between peppermint and camphor, but it can be toxic (Windholz, 1983).

The major goal of this work is to chemically characterize a mint germoplasm collection based on volatile oil constituents.

MATERIAL AND METHOD

Twenty mint genotypes obtained from Purdue University, USA collection and one Brazilian genotype (Table 1) were grown at Embrapa Genetic Resources and Biotechnology, in a randomized complete block experimental design, with five plants in each of the three replications. The above-ground biomass of each individual plant from each block was harvested from a 0.25m^2 area in November 2002, bulked, weighted, placed in a paper bag, and dried in a forced-air drier at 38^{o}C for 3 days for oil analysis. Voucher specimens of each genotype were collected and stored at the Embrapa Genetic Resources and Biotechnology herbarium (CEN). The taxonomic identification was conducted in collaboration with Dr. A. Tucker from Delaware State University. The essential oil was extracted between August and November 2003 by hydrodistillation in a modified Clevenger apparatus, in a 2 L flask during one and a half hour.

The oil was analyzed in an Agilent 6890N gas chromatograph fitted with a HP-5 (25m X 0.32mm X 0.25mm) capillary column. The oven temperature was programmed from 60ºC to 240ºC at 3ºC/min, and

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hydrogen was used as carrier gas (1.4 mL/min). Pure oil (0.1mL/1mL dichloromethane) was injected in split mode (1:100; injector at 250ºC).

Mass Spectra were obtained in an Agilent 5973N system operating in electron impact mode (EIMS) at 70 eV, coupled to an Agilent 6890 gas chromatograph fitted with a HP-5 MS column (30m X 0.25mm X 0.25mm), using the same injection procedure and oven temperature programm as above. Helium was the carrier gas, at 1.0 mL/min. The identification was based on the mass spectra of the compounds compared with the data in Wiley 6th ed. library and by their calculated retention indices (RI) compared with literature data.

TABLE 1. Essential oil content of mint germplasm accessions maintained at Embrapa Genetic Resources and Biotechnology field colletion, Brasília, DF

Accession Bra	Local Control number[1]	Common name	Scientific name[2]	Leaf dry weight yield (kg/ha)	Essential oil content (%)
000221	CM 1	Lime Mint	*Mentha aquatica* L.	3053 a-c	1.25
000230	CM 2	Apple Mint	*Mentha* x *villosa* Hudson	1260 c-e	1.47
000248	CM 3	Chocolate Mint	*Mentha* x *piperita* L.	1507 c-e	1.41
000256	CM 4	Pineapple Mint	*Mentha suaveolens* Ehrh.	1640 b-e	0.89
000264	CM 5	Chinese Mint	*Mentha canadensis* L.	3684 a-b	2.03
000281	CM 7	Grapefruit Mint	*Mentha piperita* L.	2367 a-e	2.83
000299	CM 8	Eau De Cologne	*Mentha piperita* L.	3702 a-b	1.36
000302	CM 9	Variegated Peppermint	*Mentha piperita* L.	1499 c-e	0.51
000311	CM 10	Hillary's Sweet Lemon Mint	*Mentha suaveolens* Ehrh. x *M. aquatica*	4271 a	0.76
000329	CM 11	Green Curly Mint	*Mentha piperita* L.	2257 a-e	0.78
000337	CM 13	Orange Mint	*Mentha aquatica* L.	2555 a-e	1.68
000345	CM 16	Persian Mint Field	*Mentha piperita* L.	3062 a-c	1.75
000353	CM 17	Menthol Mint Gh	*Mentha spicata* L.	1489 c-e	0.87
000361	CM 18	Common Mint Gh	*Mentha aquatica* L.	1225 c-e	1.74
000370	CM 19	Lavander Mint	*Mentha aquatica* L.	1625 b-e	1.70
000388	CM 20	Japanese Field Mint	*Mentha canadensis* L.	703 e	4.17
000400	CM 23	Peppermint	*Mentha* x *piperita* L.	1319 c-e	1.39
000418	CM 24	Ginger Mint	*Mentha arvensis* L.	575 e	0.47
000426	CM 25	Large Leaf Spearmint	*Mentha spicata* L.	1597 b-e	1.03
000451	CM 28	Egyptian Mint	*Mentha* x *villosa* Hudson	1993 b-e	1.40
000469	CM 29	Hortelã Caseira	*Mentha spicata* L.	1175 c-e	1.38

[1] CM = Mint Collection
[2]Plant identification according to Dr. Art Tucker, Dellaware State University, USA

RESULT AND DISCUSSION

This is the first report on *Mentha* spp. volatile oils cultivated at Federal District, Brazil and allowed us to identify some potential genotypes, based on agronomic (Grizi *et al*. 2003) and chemical traits. The genotypes CM1, CM5, CM7, CM8, CM10, CM11, CM13 and CM16 showed the higher yield of leaf dry weight, with no significant difference among them (Table 1). Around 67% of the accessions presented an essential oil content higher than 1%, reaching the commercial standards (Correia*, et al*., 1994). The highest volatile oil content (relative percentage of dry weight basis) was found in *Mentha canadensis* CM 20 (4.17%), *Mentha piperita* CM 7 (2.83%) and CM 5 *Mentha canadensis* (2.03%) genotypes. CM 20 genotype has showed the highest amount of menthol content, although it showed a low dry weight when comparing to the other accessions (Grizi *et al*. 2003). This study was performed during the dry season under poor fertilized soil. Since mint requires high fertilized soils and water, these results can be significantly improved during rainy season. No pulegone was found in both accessions of *M. canadensis*. However, Tucker and Chambers (2002) found most of the clones of *Mentha canadensis* had high pulegone, although menthol and menthone were also predominant constituents.

This preliminary study on *Mentha* genotypes has shown a large chemical variability among different species, and also within individuals of the same species (Table 2). The accessions studied at Brasília conditions showed fifteen major constituents (Table 2). Although several minor constituents have been presented, they are not reported in this paper.

Mentha aquatica genotypes "Lime Mint" and "Orange Mint" presented the higher leaf yield. *Mentha aquatica* genotypes have showed different oil constituents profiles. CM 1 ("Lime Mint") has presented 43.6% of limonene, CM 18 ("Common Mint") 68.8% of piperitenone oxide, CM 19 ("Lavander Mint") 44.7% of linalool and CM 13 ("Orange Mint") 35% de linalool. In Alessandra et al. (1997), *Mentha aquatica* cultivated in Italy showed low percentage of linalool and a high presence of menthofuran, 1,8-cinole, limonene and viridiflorol, as main constituents. Murray (1973) found a high percentage of menthofuran, cineole and caryophillene. These samples have not shown piperitone oxide as "Lavender Mint" showed in District Federal Brazil.

Among *Mentha piperita* genotypes, "Grapefruit" and "Eau de Cologne" presented the highest leaf yield productivity and essential oil content. These genotypes have mainly linalool and linalyl acetate, as major constituent. The highest amount of menthol was found in *Mentha piperita* varieties

TABLE 2. Major volatile oils constituents (relative percentage of essential oil) in Mint species (Mentha ssp.) growing at Federal District, Brazil.

		M. aquatica				*M. canadensis*		*M. x villosa*		*M. suavelons*	
Volatile Oil Constituents[a]	RT	CM1	CM13	CM18	CM19	CM5	CM20	CM2	CM28	CM4	CM10[b]
Limonene	10,12	43,6±2,26	8,1±7,87	1,3±2,28			1,6±0,49	9,6±2,35	14,2±0,98	1,6±0,58	7,7±2,21
1,8-cineole	10,19	16,5+2,08	3,1+5,36	5,3+6,24	3,4+1,13	6,2+0,80		3,4+0,89	3,7+0,32		5,9+0,71
Linalool	13,13		35,3±8,88		44,7±3,76					0,9±1,13	3,7±1,83
Menthone	15,57						19,3±1,77				
Neomenthol[c]	15,99										
Isomenthol[c]	16,08										
Menthol	16,58				2,1±1,57		65,0±2,79				
Dihydrocarvone	17,34							2,9±0,30	2,3±2,01		19,1±3,06
Pulegone	19,29										
Carvone	19,48	1,6±1,01		6,5±3,55	0,7±0,66			72,1±4,88	57,8±6,96	3,6±2,52	10,1±1,19
Piperitone	20,07					22,9+4,49	1,7+0,07	2,3+0,05			
Linalyl Acetate	20,24		34,8±10,25		33,7±3,58					0,4±0,72	1,0±1,07
Menthyl Acetate	22,17			0,7±1,24			4,2±0,13				
Dihydrocarvyl Acetate	23,60								1,1±0,54		14,7±1,86
Piperitenone Oxide	25,06			68,8±9,67						79,0±3,08	

[a] only volatile oil contituents higher than 10% of total oitl are reportede here.
[b] possible hybrid between *M. suave olens* x *M. aquatica.*
[c] preliminary identification.

Table 2. (Cont.)

	M. x piperita							*M. arvensis*	*M. spicata*		
Volatile Oil Constituents[a]	CM3	CM7	CM8	CM9	CM11	CM16	CM23	CM24	CM17	CM29	CM25
Limonene	1,0±0,18			2,1±0,05	4,3±0,22					7,5+2,10	5,8±1,09
1,8-cineole	4,3+0,78			1,2+1,23	14,7+1,06	50,6+6,85	3,7+0,36	0,8+1,12	10,8+0,78		6,5+1,24
Linalool	1,5±1,51	46,4±3,04	15,2±11,61		1,2±0,09			78,5±1,65	5,2±4,07	1,2+1,25	1,5±1,37
Menthone	20,6+6,10			15,2+2,40	5,2+0,38		11,4+1,51				
Neomenthol[c]	4,7±0,95			5,9±0,45	1,2±0,18		1,9±0,38		0,7±0,61		1,9±0,17
Isomenthol[c]	5,3+0,27						5,7+0,36				
Menthol	38,0±3,04			43,2±0,25	1,9±0,20		43,0±3,63				1,0±0,37
Dihydrocarvone										2,1+1,73	1,3+0,07
Pulegone				2,2+0,29		11,1+1,06	1,4+0,60				
Carvone	16,2±0,14				30,5±2,68	0,9±0,26		3,2±2,39	2,3±0,40	70,9+0,77	64,0±5,55
Piperitone				1,3+0,23							
Linalyl Acetate		41,4±1,45	19,4±15,02						1,5±1,37		
Menthyl Acetate	14,0±1,06			19,6±0,40	4,2±0,97		19,8±3,95				
Dihydrocarvyl Acetate										1,7+1,13	1,1±0,21
Piperitenone Oxide			0,5±0,44					0,5±0,75	65,5±5,91		0,5±0,85

[a] only volatile oil contituents higher than 10% of total oitl are reportede here.
[b] possible hybrid between *M. suave olens* x *M. aquatica.*
[c] preliminary identification.

"Chocolate Mint" (38.0 %), "Variegated Peppermint" (43.2%), "Peppermint" (43.0 %) and *Mentha canadensis* variety "Japanese Field Mint" (65.0 %). Among the other varieties of *Mentha piperita,* "Green Curly Mint" presented 30.5 % of carvone, and "Persian Mint Field" 50.6 % of 1,8-cineole.

The varieties of *Mentha villosa* CM 2 ("Apple Mint") and CM 28 ("Egyptian Mint") has presented, respectively, 72.1 % and 57.8 % of carvone. "Egyptian Mint" presented the highest leaf production in District Federal region.

The varieties of *Mentha spicata* CM 17 ("Menthol Mint GH") has presented high content of piperitenone oxide (65.5 %), and CM 25 ("Large Leaf Spearmint") and CM 29 ("Hortelã caseira") showed 64.0% and 70.9% of carvone, respectively. The CM 29 genotype is largely used in Brazilian-Arabian culinary and also as a phytomedicine. Soliman et al. (1997) found a high amount of pulegone (42.54) and 1,8-cineole (13.45) in *Mentha spicata* from Cairo University.

Mentha suavelons ("Pineapple Mint") presented low amount of essential oil and 79% of piperitone oxide.

The *Mentha arvense* genotype CM 24 ("Ginger Mint") showed higher linalool content (78.5%), and the lowest leaf produtivity and essential oil content.

A further study of each species at rainy season will be conducted to better understand the mint essential oil production at Brasilia environmental conditions.

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