

A survey of essential oils from *Mentha* spp. as an antimicrobial potential agent against *Candida* species

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ABSTRACT

Mentha spp. essential oils have shown different properties regarding accession despite belonging to the same species and antimicrobial properties indicating possible clinical relevance. A survey of 64 accessions of *Mentha* spp. essential oils were carried out proving their differences regarding yield and potential antimicrobial activity against *Candida albicans*, *Candida dubliniensis* and clinical samples. The oils were obtained by water vapor-distillation by cleverger. Anti-*Candida* activity was assessed through the MIC (minimal inhibitory concentration) of the plants. The oils that showed better activity were selected for chemical characterizations analyzed through Gas Chromatographic and mass spectrometry (GC-MS) and biofilm assays in formation and mature biofilm. The best activity against planktonic cells of *Candida* spp. were *M. canadensis* MC 05; *M. suaveolens* MC 30; *M. arvensis* MC 36 and *M. suaveolens* x *spicata* MC 52. These oils revealed inhibition against yeast cells and promoted the best MIC (≤ 0.5 mg/ml). MC 05 was enriched with carvone (74.8%); MC 30 with piperitenone oxide (28.0%); MC 36 with linalool (33.2%) and MC 52 with pulegone (52.2%). All the essential oils showed action on the biofilm in formation and mature biofilm, being that the formation of biofilm was inhibited up to 96% and the mature biofilm was deconstructed up to 54%. The results obtained from these essential oils revealed genotypes with different properties and anti-*Candida* activity, a number of these oils could contribute to the characterization of the antimicrobial properties due to their phenotypical characterization.

Keywords: *Candida* spp., essential oil, medicinal plants, *Mentha* spp.

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INTRODUCTION

Essential oils and isolated compounds from medicinal plants have demonstrated inhibition against bacteria, yeast and filamentous fungi (Sartoratto et al., 2004; Duarte et al., 2005; Zapata et al., 2010; Furletti et al., 2011). These antimicrobial properties of the medicinal plants indicate their possible clinical relevance (Kim et al., 2008).

There are some investigations regarding the different properties of the same species of *Mentha* species. This genus belongs to Lamiaceae family and is known to have

more than 25 species in the world. The properties of *Mentha* are anti-inflammatory, antiviral and antispasmodic activities. Many species of *Mentha* have shown antimicrobial activities according to *in vitro* studies (Mimica-Dukic and Bozin, 2008; Sartoratto et al., 2004). Reports in the literature have described *Mentha* spp. as being active against *C. albicans* (Sartoratto et al., 2004; Tampieri et al., 2005; Höfling et al., 2010). The essential oil from *Mentha* spp. has been evaluated by some authors (Nair, 2001; McKay and Blumberg, 2006; Gurib-

Fakin, 2006; Bush et al., 2007; Hur et al., 2007), due to the use of this plant in the pharmacological industry (Oumzil et al., 2002; McKay and Blumberg, 2006), and cosmetology industry (Bhatia et al., 2008). However, this genus is very important as raw material for development of new therapeutic products. The effects of *Mentha* spp. essential oil upon potential pathogenic fungi in humans, such as *Candida* spp., have been studied and have revealed different properties despite belonging to the same species.

The frequency of *Candida albicans* infection has increased with the emergence of strains resistant to the antifungal drugs currently in use (Niimi et al., 2010). *Candida* spp. are opportunistic pathogens that can cause local and systemic infections in immunologically compromised patients and individuals undergoing prolonged antibiotic treatment (Dahlén et al., 2009). *C. albicans* is the most common species associated with candidiasis; the fourth leading blood-borne infection, with mortality rates as high as 47% (Wilson et al., 2002). *C. albicans* forms biofilms that are attached to mucosal surfaces, these biofilms attach to implanted medical devices, such as intravenous catheters, shunts, stents, and oral biofilm (Prashar et al., 2003; Nogueira et al., 2008).

There are other *Candida* spp. related to candidiasis, such as *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata* and *Candida dubliniensis* (Xu et al., 1999; Badiie et al., 2010). *C. dubliniensis* has also been isolated from the respiratory tract, vagina, and gastrointestinal tract, in addition to urine, sputum, and wounds, and is implicated in invasive infections, including HIV-negative individuals, suffering from serious diseases (Jainkittivong et al., 1998; Fotedar and Al-Hedaithy, 2004; Sullivan et al., 2005) and is generally associated with *C. albicans* and/or other species of the genus (Perea et al., 2001).

In the present study the antifungal activity, *in vitro*, were analyzed for 64 genotypes and species of the *Mentha* for collection of the medicinal and aromatic plants of CPQBA-UNICAMP, with the purpose of identifying that species tested exhibit more activity against strains *Candida* spp. The oils of species that showed more activity were test against biofilm of *Candida albicans*. The chromatographic profiles were identified by gas chromatography/mass spectrometric analyses to identify their major compounds.

METHODOLOGY

Medicinal plants and essential oil extraction

Sixty-four species of plants were collect from different locations and grown at the experimental field of the Research Center for Chemistry, Biology and Agriculture (CPQBA), University of Campinas, São Paulo, Brazil (Table 1). The exsicate of the plant

material selected for this study was deposited at the herbarium of the Brazilian Center of Biotechnology and Genetic Resources CENARGEN/EMBRAPA, Brasília, DF, or at the Aromatic and Medicinal Plant Collection -CPMA, Paulínia, SP, Brazil.

The essential oils were obtained from 200 g of fresh plant parts by water-distillation using a Clevenger-type system for 3 h. The aqueous phase was extracted three times with 50 ml dichloromethane. The pooled organic phases were dried with sodium sulphate, filtered and the solvent evaporated until dry. Oil samples were stored at -25°C in sealed glass vials.

Microorganisms

Reference strains of two species of *Candida* spp., were used from the collection of the Dutch CBS (CBS 7987- *Candida dubliniensis* and CBS 562 *Candida albicans*), and seven clinical isolates from patients with periodontal disease, systemically healthy, were collected at the periodontal clinical, Piracicaba Dental School / UNICAMP (Table 2), and tested, the result showed 4 *Candida albicans* species and 3 *Candida dubliniensis* species. This study was approved by the Ethics Review Board 060/2008.

Planktonic anti-Candida assay – (CLSI, 2002)

The 64 essential oils were tested for antimicrobial activity against planktonic *Candida* spp. The yeast was grown overnight at 37°C in Sabouraud Dextrose Agar (Merck®) plates, and the inoculum for the assays was prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted to McFarland scale 0.5 and confirmed by spectrophotometric reading at 580 nm. Cell suspensions were finally diluted to 10⁴ UFCml⁻¹ for use in assays. MIC tests were carried out according to (CLSI, 2002), using a test plate (96 wells). The stock solutions of the oils were diluted and transferred into the first well, and serial dilutions were performed in sequence where concentrations in the range of 1 to 0.007 mgml⁻¹ were obtained. Nistatin (Merck®) was used as the reference antimycotic control in the range of 5 to 60 gml⁻¹. The yeast inoculum was added to all wells and the plates were incubated at 36°C for 48 h. MIC was defined as the lowest concentration of oil and extract that inhibited visible growth.

Biofilm assay

The oils essential for showed better activity in test planktonic cells of *Candida* spp. were tested against formation biofilm and mature biofilm of *Candida albicans* (SC 5314).

Preparation of inoculum

The yeast strain were incubating overnight at 37°C in SDA plate, a loopful from these cells were inoculated in 50 ml of YPD liquid medium and grown overnight in an orbital shaker (150 to 180 rpm) at 30°C. The cells were washed twice with sterile PBS, centrifuged at 3000 g, and were resuspended in RPMI. Posteriorly, the cells were count using a haemocytometer on a bright field microscope (Dilutions 1:100 in PBS). The concentration of inoculum was in 1.0 × 10⁶ cells/ml.

Biofilm formation

In a microtiter plate pre-sterilized, polystyrene, flat-bottomed, 96-

Table 1. Identification and origin of the plants studied.

Voucher no.	Local code	Popular name	Botanical name*	Origin
CEN47633	MC 1	Lime mint	<i>Mentha aquatica</i> L.	Univ. Purdue USA
CEN47634	MC 2	Apple mint	<i>Mentha x villosa</i> Huds.	Univ. Purdue USA
CEN 47635	MC 3	Chocolate mint	<i>Mentha x piperita</i> L.	Univ. Purdue USA
CEN 47636	MC 4	Pineapple mint	<i>Mentha suaveolens</i> Ehrh.	Univ. Purdue USA
CEN 47637	MC 5	Chinese mint	<i>Mentha canadensis</i> L.	Univ. Purdue USA
CEN 47638	MC 6	Chewing gum mint	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47640	MC 8	Eu' de cologne	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47641	MC 09	Variegated peppermint	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47642	MC 10	Hillary's sweet lemon mint	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47643	MC 11	Green curly mint	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47644	MC 13	Orange mint	<i>Mentha suaveolens</i> Ehrh.	Univ. Purdue USA
CEN 47646	MC 16	Persian mint field	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47647	MC 17	Menthol mint gh	<i>Mentha spicata</i> L.	Univ. Purdue USA
CEN 47648	MC 18	Common mint gh	<i>Mentha aquatica</i> L.	Univ. Purdue USA
CEN 47649	MC 19	Lavander mint	<i>Mentha aquatica</i> L.	Univ. Purdue USA
CEN 47650	MC 20	Japanese field mint	<i>Mentha canadensis</i> L.	Univ. Purdue USA
CEN 47651	MC 22	Bergamot	<i>Mentha x gracilis</i> Sole	Univ. Purdue USA
CEN 47652	MC 23	Peppermint	<i>Mentha x piperita</i> L.	Univ. Purdue USA
CEN 47653	MC 24	Ginger mint	<i>Mentha arvensis</i> L.	Univ. Purdue USA
CEN 47654	MC 25	Large leaf spearmint	<i>Mentha spicata</i> L.	Univ. Purdue USA
CEN 47655	MC 26	Banana mint	<i>Mentha piperita</i> L.	Univ. Purdue USA
CEN 47656	MC 27	Himalayan silver mint	<i>Mentha longifolia</i> (L.) Huds.	Univ. Purdue USA
CEN 47657	MC 28	Egyptian mint	<i>Mentha</i> sp. x <i>M. Villosa</i> Huds.	Univ. Purdue USA
CEN 47658	MC 29	Hortelã caseira	<i>Mentha spicata</i> L.	Distrito Federal
CEN 69183	MC 30	Menta do Uruguai	<i>Mentha suaveolens</i> Ehrh.	Uruguai
CPMA1927	MC 31	Emater 1	<i>Mentha spicata</i> L.	UnB
CPMA1928	MC 32	Ciudad de leste	<i>Mentha spicata</i> L.	UnB
CPMA1929	MC 33	UnB1	<i>Mentha spicata</i> L.	UnB
CPMA1930	MC 34	Emater 2	<i>Mentha sylvestris</i> L.	UnB
CPMA1931	MC 35	Dourados 1	<i>Mentha spicata</i> x <i>suaveolens</i>	UnB
CPMA1932	MC 36	Dourados 2	<i>Mentha arvensis</i> L.	UnB
CEN69184	MC 37	Emater 3	<i>Mentha canadensis</i> L.	UnB
BRA000558	MC 38	Kibe	<i>Mentha spicata</i> x <i>suaveolens</i>	UnB
BRA000566	MC 39	Piperita negra	<i>Mentha longifolia</i> L. Huds./ <i>Mentha spicata</i> L.	UnB
BRA000574	MC 40	Hortelã branca	<i>Mentha longifolia</i> (L.) Huds.	UnB
CEN69182	MC 41	Hortelã vilosa	<i>Mentha suaveolens</i> Ehrh.	CPQBA
CPMA560	MC 43	Hortelã	<i>Mentha piperita</i> L.	CPQBA
CPMA489	MC 44	Hortelã	<i>Mentha x piperita</i> subsp. <i>Citrata</i> Ehrh.	CPQBA
CPMA500	MC 45	Hortelã branca	<i>Mentha gracilis</i>	CPQBA
CPMA403	MC 46	Hortelã	<i>Mentha arvensis</i> L.	CPQBA
CEN69186	MC 47	Hortelã 1802	<i>Mentha piperita</i> L. var <i>citrata</i>	São Paulo
CPMA1937	MC 49	IAC 1	<i>Mentha gracilis</i>	IAC
CEN69187	MC 50	IAC 2	<i>Mentha spicata</i> L.	IAC
CPMA1939	MC 51	IAC 3	<i>Mentha suaveolens</i> Ehrh. x <i>spicata</i>	IAC
CPMA1940	MC 52	IAC 4	<i>Mentha suaveolens</i> Ehrh. x <i>spicata</i>	IAC
CPMA1941	MC 53	IAC 5	<i>Mentha spicata</i> L.	IAC
CEN69176	MC 54	IAC 6	<i>Mentha spicata</i> L.	IAC
CPMA1943	MC 55	IAC 7	<i>Mentha spicata</i> L.	IAC
CEN69177	MC 56	IAC 8	<i>Mentha piperita</i> L. var <i>citrata</i>	IAC

Table 1. Continues.

CPMA1945	MC 57	IAC 9	<i>Mentha piperita</i> L.	IAC
CEN69181	MC 58	Hortelã feltrini	<i>Mentha spicata</i> L.	Sementes comerciais
CPMA1947	MC 59	Iac 701	<i>Mentha arvensis</i> L.	Paraná
CPMA1948	MC 60	K 78195	<i>Mentha aquatica</i> L.	Kew Garden Inglaterra
CPMA1949	MC 61	K 69652	<i>Mentha suaveolens</i> Ehrh.	Kew Garden Inglaterra
CEN69179	MC 62	Hocaido	<i>Mentha arvensis</i> L.	Paraná
CPMA1951	MC 63	Poejo botucatu	<i>Mentha pulegium</i>	UNESP
CEN69179	MC 64	Hortelã botucatu	<i>Mentha spicata</i> L.	UNESP
CPMA1953	MC 65	UFC 1	<i>Mentha</i> sp. x <i>M. villosa</i> Huds.	UFC
CEN69180	MC 66	UFC 2	<i>Mentha gracilis</i>	UFC
CPMA1955	MC 67	UFC 3	<i>Mentha x piperita</i> subsp. <i>Citrata</i> Ehrh.	UFC
CPMA1956	MC 68	UFC 4	<i>Mentha rotundifolia</i> (L.) Huds.	UFC
CPMA1957	MC 69	UFC 5	<i>Mentha piperita</i> L.	UFC
CPMA1958	MC 70	CA 1	<i>Mentha suaveolens</i> Ehrh.	Minas Gerais
CPMA1960	MC 72	CA 3	<i>Mentha spicata</i> L.	Minas Gerais

Table 2. *Candida* spp. from the codes collection and clinical isolates studied.

<i>Candida</i> spp.	Code	Clinical Isolates
<i>C. albicans</i>	CBS-562	3A5; 13A5; 15A5; 47A5
<i>C. dubliniensis</i>	CBS-7987	26A2; 26A3; 26A4

well, were inoculated 100 µl to RPMI with oils essential at the pre-established concentration and 100 µl to inoculum adjusted. The plates were incubated at 37°C for 24 h.

Mature biofilm

In a microtiter plate with same specifications were inoculated 100 µl to inoculum adjusted and were incubate at 37°C for 24 h. Then, the plates were washed 3x with sterile PBS and added 100 µl to RPMI with oils essential in the pre-established concentration. The plates were incubated at 37°C for 24 h.

Biofilm quantification

After incubation, the plates were washed 3x with sterile PBS for remove planktonic cells. The semi-quantification of the fungal cell viability was calculate using a colorimetric XTT reduction assay, in which were add in the plates 80 µl solution XTT and the absorbance measurement above in reader microtiter spectrophotometer at 490 nm.

Gas chromatographic (GC) and mass spectrometry (GC-MS) analyses

The identification of volatile constituents from *Mentha* spp. essential oil that presented best antimicrobial activity was performed using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5971 mass selective detector and capillary column HP-5 (25 m × 0.2 mm × 0.33 m diameter). GC and GC-MS were performed

using split/splitless injection, with injector set at 220°C, column set at 60°C, with heating ramp of 3°C min⁻¹ and final temperature of 240°C for 7 min, and the FID detector set up at 250°C. Helium was used as the carrier gas at 1 mlmin⁻¹. The GC-MS electron ionization system was set up at 70 eV. A sample of the essential oil was solubilized in ethyl acetate for the analyses. Retention indices (RI) were determined by co-injection of hydrocarbon standards. Oil components were identified by comparison with data from the literature, the profiles from the Wiley 138 and Nist 98 libraries, and by co-injection of authentic standards, when available.

RESULTS

Essential oil

The oil yields of the plants indicated in Table 1 are presented in Table 3. The results were expressed in relation to dry weight of plant material (% w/w). The largest quantities of essential oil were obtained from *M. spicata* (MC 31) (1.50%) and *Mentha* sp. (MC 53) (1.45%) and the lower quantities were obtained from *M. rotundifolia* (CM 35) (0.03%) and *M. piperita* L. (CM 69) (0.04%).

Essential oil antimicrobial activity against planktonic *Candida* spp.

Minimum Inhibitory Concentration (MIC) of the essential oils (EO) was tested against *Candida* spp. planktonic cells, as indicated in Table 4. In general, the oils presented action against planktonic *Candida* spp. and clinical isolates ≥ 1 mg/ml; however there were some oils incapable of inhibiting the strains at MIC values up to 0.500 mg/ml (EO -Table 4).

M. aquatica L. (CM 19) for the clinical isolate 13A5

Table 3. Essential oil (EO) yields from the medicinal plants studied.

<i>Mentha</i> spp. (EO)	Origin	Code	Dry plant mass (g)	Oil yield dry basis (%)	Humidity (%)
<i>M. aquatica</i> L.	USA	MC 01	0.4	0.1	82.0
<i>Mentha</i> spp. <i>M. villosa</i> Huds	USA	MC 02	0.3	0.2	89.0
<i>Mentha x piperita</i> L.	USA	MC 03	0.1	0.1	89.0
<i>M. suaveolens</i> Ehrh	USA	MC 04	0.2	0.8	89.0
<i>M. canadensis</i> L.	USA	MC 05	0.3	0.9	73.0
<i>M. piperita</i> L.	USA	MC 06	0.2	1.2	91.0
<i>M. piperita</i> L.	USA	MC 08	0.3	0.6	89.0
<i>M. piperita</i> L.	USA	MC 09	0.2	0.4	90.0
<i>M. suaveolens x aquatica</i>	USA	MC 10	0.3	0.8	83.0
<i>M. piperita</i> L.	USA	MC 11	0.2	0.8	83.0
<i>M. aquatica</i> L.	USA	MC 13	0.1	0.1	93.0
<i>M. piperita</i> L.	USA	MC 16	0.3	0.7	87.0
<i>M. spicata</i>	USA	MC 17	0.2	0.2	87.0
<i>M. aquatica</i> L.	USA	MC 18	0.4	0.1	82.0
<i>M. aquatica</i> L.	USA	MC 19	0.3	0.8	90.0
<i>M. canadensis</i>	USA	MC 20	0.5	1.0	82.0
<i>Mentha x gracilis</i> Sole	USA	MC 22	0.2	0.2	83.0
<i>Mentha x piperita</i> L.	USA	MC 23	0.2	0.05	88.0
<i>M. arvensis</i> L.	USA	MC 24	0.2	0.95	91.0
<i>M. spicata</i> L.	USA	MC 25	0.2	0.19	89.0
<i>M. arvensis</i> L.	USA	MC 26	1.1	0.15	46.0
<i>M. longifolia</i>	USA	MC 27	0.4	0.23	82.0
<i>Mentha</i> spp. <i>M. villosa</i> Huds	USA	MC 28	0.30	0.40	86.0
<i>M. spicata</i> L.	DF	MC 29	0.20	0.30	92.0
<i>Mentha</i> sp.	Uruguai	MC 30	0.30	0.20	85.0
<i>M. spicata</i> L.	UnB	MC 31	0.30	1.50	85.0
<i>M. piperita</i> L.	UnB	MC 32	0.30	0.05	82.0
<i>M. spicata</i> L.	UnB	MC 33	0.25	0.30	80.0
<i>M. sylvestris</i>	UnB	MC 34	0.20	0.30	85.0
<i>M. rotundifolia</i> L.	UnB	MC 35	0.20	0.03	92.0
<i>M. arvensis</i> L.	UnB	MC 36	0.40	0.50	86.0
<i>M. campestris</i> Shur	UnB	MC 37	0.40	1.30	87.0
<i>Mentha</i> sp. <i>M. villosa</i> Huds	UnB	MC 38	0.25	0.30	90.0
<i>M. piperita</i> L.	UnB	MC 39	0.25	0.85	88.0
<i>M. longifolia</i> L. Huds	UnB	MC 40	0.40	0.15	80.0
<i>M. spicata</i> L.	CPQBA	MC 41	0.20	0.80	90.0
<i>M. piperita</i> L.	CPQBA	MC 43	0.35	0.10	85.0
<i>M. piperita /citrate</i>	CPQBA	MC 44	0.30	0.35	85.0
<i>M. suaveolens</i>	CPQBA	MC 45	0.15	0.30	92.0
<i>M. arvensis</i> L.	CPQBA	MC 46	0.65	1.00	79.0
<i>Mentha</i> sp.	SP	MC 47	0.48	0.10	76.0
<i>Mentha</i> sp.	IAC	MC 49	0.30	0.15	87.0
<i>Mentha</i> sp.	IAC	MC 50	0.20	0.40	89.0
<i>Mentha</i> sp.	IAC	MC 51	0.25	0.10	88.0
<i>Mentha</i> sp.	IAC	MC 52	0.30	0.00	90.0
<i>Mentha</i> sp.	IAC	MC 52	0.40	0.20	80.0
<i>Mentha</i> sp.	IAC	MC 53	0.65	0.00	68.0
<i>Mentha</i> sp.	IAC	MC 53	0.20	1.45	90.0
<i>Mentha</i> sp.	IAC	MC 54	0.20	0.35	92.0

Table 3. Continues.

<i>Mentha</i> sp.	IAC	MC 55	0.10	0.55	92.0
<i>Mentha</i> sp.	IAC	MC 56	0.45	0.10	88.0
<i>Mentha</i> sp.	IAC	MC 57	0.30	0.10	87.0
<i>Mentha</i> sp.	SC	MC 58	0.10	0.15	90.0
<i>M. arvensis</i> L.	Paraná	MC 59	0.40	0.40	80.0
<i>M. aquatica</i> L.	KG	MC 60	0.30	1.30	86.0
<i>M. suaveolens</i> Ehrh	KG	MC 61	0.30	0.10	88.0
<i>Mentha</i> sp.	Paraná	MC 62	0.45	0.00	80.0
<i>Mentha</i> sp.	Paraná	MC 62	0.15	0.85	90.0
<i>Mentha</i> sp.	Unesp	MC 63	0.25	0.10	89.0
<i>Mentha</i> sp.	Unesp	MC 64	0.20	0.50	92.0
<i>Mentha</i> sp <i>M villosa</i> Huds	UFC	MC 65	0.20	0.00	87.0
<i>Mentha</i> sp <i>M villosa</i> Huds	UFC	MC 65	0.25	0.05	88.0
<i>M. arvensis</i> L.	UFC	MC 66	0.25	0.20	87.0
<i>M. piperita/citrata</i> Ehrh	UFC	MC 67	0.45	0.75	89.0
<i>M. rotundifolia</i> Huds	UFC	MC 68	0.40	0.35	90.0
<i>M. piperita</i> L.	UFC	MC 69	0.20	0.00	89.0
<i>M. piperita</i> L.	UFC	MC 69	0.20	0.05	90.0
<i>Mentha</i> sp.	MG	MC 70	0.35	0.00	83.0
<i>Mentha</i> sp.	MG	MC 70	0.30	0.10	89.0
<i>Mentha</i> sp.	MG	MC 72	0.35	0.15	88.0

Table 4. Anti-*Candida* activity (MIC - mg/ml) of the essential oils from the medicinal species studied.

<i>Candida</i> /MC	Ca 3A5	Ca 13A5	Ca 15A5	Ca 47A5	Ca CBS	Cd 26A4	Cd 26A3	Cd 26A2	Cd CBS
01	*	*	*	*	*	*	*	*	*
02	*	*	*	*	*	*	*	*	*
03	*	*	*	*	*	*	*	*	*
04	*	*	*	*	*	*	*	*	*
05	0.031	0.015	0.031	0.125	<0.007	0.187	<0.007	0.031	0.031
06	*	*	*	*	*	*	*	*	*
08	*	*	*	*	*	*	*	*	*
09	*	*	*	*	*	*	*	*	*
10	*	*	*	*	*	*	*	*	*
11	*	*	*	*	*	*	*	*	*
13	*	*	*	*	*	*	*	*	*
17	*	*	*	*	*	0.250	0.250	0.500	0.187
18	*	*	*	*	*	*	*	*	*
19	*	0.187	*	*	*	1.000	*	1.000	0.312
20	*	*	*	*	*	*	*	*	*
22	*	*	*	*	*	*	*	*	*
23	*	*	*	*	*	0.500	1.000	1.000	0.500
24	*	*	*	*	*	*	*	*	*
25	*	*	*	*	*	*	*	*	*
26	*	*	*	*	*	1.000	*	*	1.000
27	0.046	*	*	*	*	*	*	*	*
28	*	*	*	*	*	*	*	*	*
29	1.000	1.000	1.000	1.000	1.000	0.062	0.125	0.125	0.062
30	0.500	0.500	0.500	0.500	0.500	0.046	0.062	0.125	0.062

Table 4. Continues.

31	*	*	*	*	*	*	*	*	*
32	*	*	*	*	*	*	*	*	*
33	*	*	*	*	*	0.187	*	1.000	0.750
34	*	*	*	*	*	0.125	*	*	0.750
35	*	*	*	*	*	*	*	*	*
36	*	<0.007	1.000	1.000	0.250	0.125	0.250	0.250	0.312
37	*	*	*	*	*	0.187	*	*	1.000
38	*	*	*	*	*	0.500	*	*	*
39	*	*	*	*	*	*	*	*	*
40	*	*	*	*	*	*	*	*	*
41	*	*	*	*	*	*	*	*	*
43	*	*	*	*	*	*	*	*	*
44	*	*	*	*	*	*	*	*	*
45	*	*	*	*	*	*	*	*	*
46	*	*	*	*	*	*	*	*	0,750
47	*	*	*	*	*	*	*	*	*
49	*	*	*	*	*	*	*	*	*
50	*	*	*	*	*	*	*	*	*
51	0.125	*	*	*	*	*	*	*	*
52	0.500	0.500	0.500	0.500	0.250	0.062	0.062	0.125	0.062
53	*	*	*	*	*	*	*	*	*
54	*	*	*	*	*	*	*	*	*
55	*	*	*	*	*	*	*	*	*
56	0.125	*	*	*	*	*	*	*	*
57	*	*	*	*	*	*	*	*	*
58	*	*	*	*	*	*	*	*	*
59	0.125	*	*	*	*	*	*	*	*
60	*	*	*	*	*	*	*	*	*
61	*	*	*	*	*	*	*	*	*
62	*	*	*	*	*	*	*	*	*
63	*	*	*	*	*	*	*	*	*
64	*	0.031	*	0.125	0.046	*	< 0.007	0.125	0.125
65	*	*	*	*	*	*	*	*	*
66	*	*	*	*	*	*	*	*	*
67	*	*	*	*	*	*	*	*	*
68	*	*	*	*	*	*	*	*	*
69	*	*	*	*	*	*	*	*	*
70	<0.007	*	*	*	*	*	*	*	*
72	0.015	*	*	*	*	*	*	*	*

* = > 1 mg/ml, C.a. = *Candida albicans*, C.d. = *Candida dubliniensis*.

(0.1875 mg/ml) and CBS 7987 (0.312 mg/ml), as occurred with *Mentha X piperita* (CM 23) for 13A5 (0.500 mg/mL) and CBS 7987 (0.500 mg/ml); *Mentha* spp. *M villosa* Huds (CM 38) (0.500 mg/ml), *M. campestris* Shur (CM 37) (0.1875 mg/ml) and *M. spicata* L. (CM 33) (0.1875 mg/ml) for the clinical isolate 26A4; *M. silvestris* L. (CM 34) (0.125 mg/ml) for CBS 7987; *M. spicata* (CM 17) for all strains of *C. dubliniensis* with MIC values ranging from 0.1875 mg/mL to 0.500 mg/mL; *Mentha* sp.

(CM 72) (0.015 mg/ml), *Mentha* sp. (CM 70) (0.007 mg/ml), *M. longifolia* (CM 27) (0.046 mg/ml), *M. arvensis* L. (CM 59) (0.125 mg/ml), *Mentha* sp. (CM 56) (0.125 mg/ml) and *Mentha* sp. (CM 51) (0.125 mg/ml) only for the clinical isolate 3A5; *Mentha* sp. (CM 64) for strains 13A5 (0.031 mg/ml), 47A5 (0.125 mg/ml), CBS 562 (0.046 mg/ml), 26A3 (<0.750 mg/ml), 26A2 (0.125 mg/ml), CBS 7987 (0.125 mg/ml); *M. arvensis* L. (CM 36) for strains CBS 562 (0.250 mg/ml), 13A5 (< 0.007 mg/ml)

Table 5. Anti-*Candida* activity (MIC – mg/ml) of antifungal drugs.

<i>Candida</i> spp.	CBS-562	3A5	13A5	15A5	47A5	CBS-7987	26A2	26A3	26A4
MIC (µg/ml)	5.2	5.2	5.2	7.8	6.5	6.5	3.9	5.2	7.8

and all strains of *C. dubliniensis* (between 0.125 and 0.312 mg/ml).

In addition to CM 36, 3 other accesses were noteworthy, presenting a strong activity of broad spectrum, inhibiting all strains tested, which were: *M. canadensis* (CM 05) - *C. dubliniensis* (<0.007 to 0.187 mg/ml); and *C. albicans* (<0.007 to 0.500 mg/ml); *Mentha* sp. (CM 30) - *C. dubliniensis* (0.062 to 0.125 mg/ml); *C. albicans* (0.500 mg/ml) and *Mentha* spp. (CM 52) - *C. dubliniensis* (0.062 to 0.125 mg/ml); *C. albicans* (0.250 to 0.500 mg/ml).

A commercially available antifungal agent was chosen, Nistatina (Merck® polienic - fungicide), to compare the results of inhibition obtained using the medicinal plants. MIC results are presented in Table 5.

Gas chromatographic (GC) and mass spectrometry (GC-MS) analyses

The chemical composition of the 4 essential oils obtained from *Mentha* spp. that presented best antimicrobial activity are shown in Figure 1, and Tables 6, 7, 8 and 9. The evaluation was performed by gas chromatography coupled to mass spectrometry (GC-MS). Oil analysis indicated the presence of volatile derivatives (terpenes) of alcohols and aldehydes, such as menthone, menthol, linalool, carvone, limonene, piperitenone, gamma muurolene, pulegone (Figure 1):

- i) *Mentha canadensis* CM 05 was enriched with carvone (75.0%), linalool (4.5%), dihydro carveol acetate (2.0%);
- ii) *Mentha suaveolens* CM 30 was enriched with piperitenone oxide (28.0%), E-beta-farnesene (18.5%), gamma-muurolene (17.5%);
- iii) *Mentha arvensis* CM 36 was enriched with linalool (33.0%), linalool acetate (22.0%), alpha-terpineol (12.0%);
- iv) *Mentha suaveolens x spicata* CM 52 was enriched with pulegone (52.0%), piperitenone (29.5%), gamma-muurolene (4.5%).

Biofilm assay

Essential oils of access CM05, CM30, CM36 and CM52 showed activity against biofilm formation of *Candida albicans* at the following concentrations: CM 05- *M. canadensis* inhibited by 78% (8 mg/ml), 52% (4 mg/ml)

from 2 mg/ml inhibition was below 15%; CM 30- *M. spicata*, 93% (8 mg/ml), 79% (4 mg/ml), 53% (2 mg/ml), and from 1 mg/ml inhibition was below 1%; CM36- *M. arvensis*, 96% (8 mg/ml), 89% (4 mg/ml), and from 2 mg/ml was below 59%; CM *suaveolens x 52- M. spicata* 97% (8 mg/ml), 86% (4 mg/ml), 70% (2 mg/ml), 45% (1 mg/ml) and below 0.5 mg/ml was below 1%. The results for the test with conventional antifungal (fluconazole), showed inhibition up to 64% (0.03 µg/ml); Amphotericin B at 75% (1 µg/ml) and from 0.5 µg/ml showed inhibition below 58%.

Only essential oils extracted from *Mentha* spp. access the CM 30 and CM 52 caused deconstruction of biofilm up to 50% in mature biofilms of *C. albicans* (SC 5314). The action of CM 05- *M. canadensis* was 15% deconstruction in the concentration of 8 mg/ml and below 7% for other concentrations; CM 30- *M. spicata*, 53% (8 mg/ml) and below 31% for all other concentrations; CM36- *M. arvensis*, 15% at concentration of 8 mg/ml, below 7% for all other concentrations; CM 52- *M. suaveolens x spicata*, 54% (8 mg/ml) and below 35% for all other concentrations. For conventional antifungal Fluconazole was 18% (16 µg/ml) and below 18% for all other concentrations; Amphotericin B, 62% (1 µg/ml) below 42% in others concentrations.

DISCUSSION

The most consumed essential oils are those from the *Mentha* species and have proven antimicrobial activity throughout the World (Oumzil et al., 2002; McKay and Blumberg, 2006) and the oil derivatives are broadly used in cosmetics, hygiene and food products (Nair, 2001). To select the accessions with anti-*Candida* properties and optimize both industrial use and genetic improvement, 64 different *Mentha* accessions were tested, based on yield, antimicrobial properties and chromatographic analysis. The different species of *Mentha* collected and herein studied for their antimicrobial activity, although cultivated at CPMA – CPQBA/UNICAMP (Collection of Medicinal and Aromatic Plants of CPQBA/UNICAMP) (Table 1) were obtained from different accessions. The data regarding plant collection and plant mass used, yields and humidity rates are presented in Table 3.

Despite the differences in yield of the essential oils, the different *Mentha* ssp. accessions tested showed a low yield 0.30 and 1.5 % (dry weight basis). These results are in accordance with the studies of Deschamps et al. (2008),

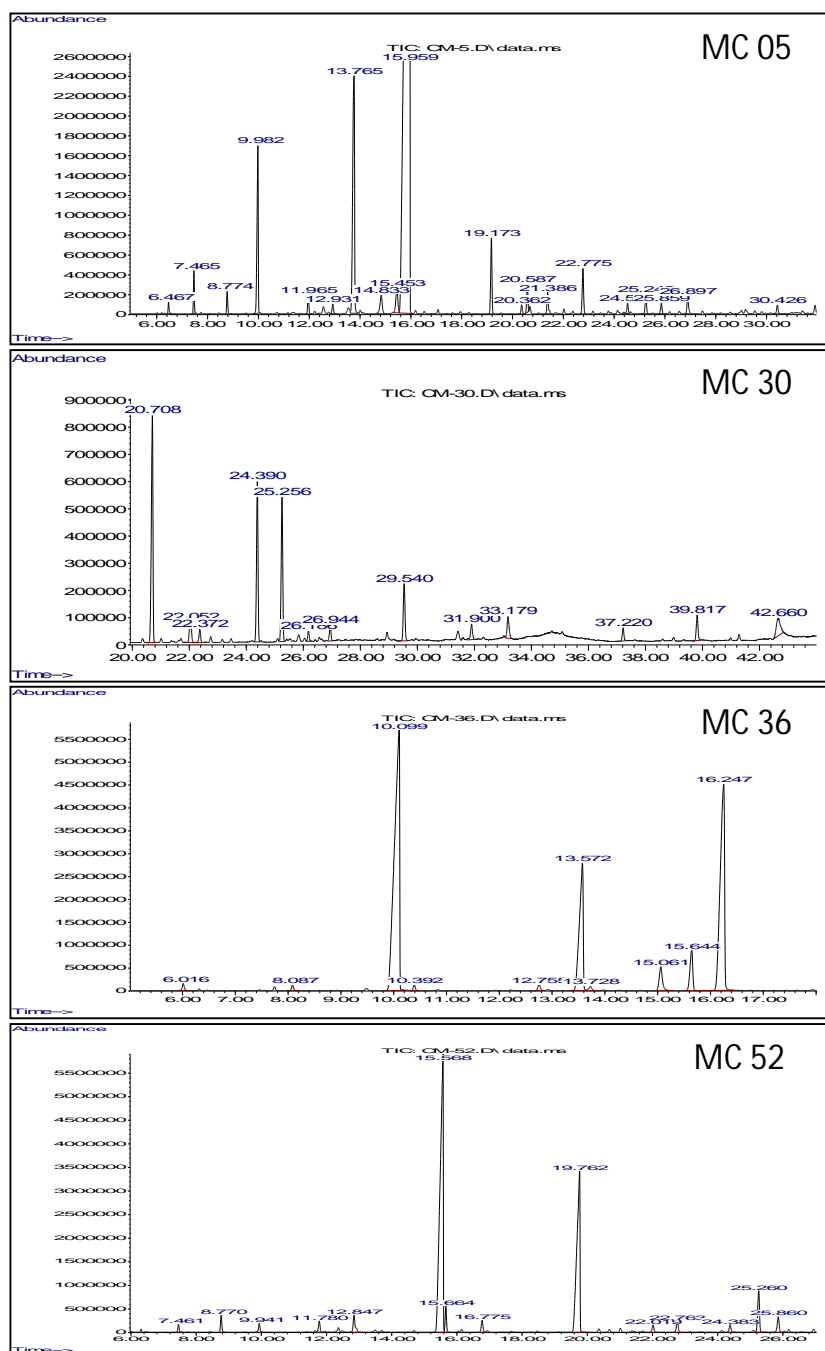


Figure 1. Chromatogram (GC-MS analysis) of the essential oil from *M. canadensis* MC 05, *M. suaveolens* Ehrh MC 30, *M. arvensis* L. MC 36 and *M. suaveolens* Ehrh. x *spicata*.

which demonstrated a great variability of yields (from 0.15 to 0.7%), suggesting that the biosynthesis of the essential oils could result from the physiological and genetic characteristics of the plant, as well as to climate, temperature, and exposure to radiation.

The results obtained herein confirm that different accessions of a same species of *Mentha* can present different physiological properties, as suggested by several studies (Spirling and Daniels, 2001; Xu et al., 2003; Mahboubi and Haghi, 2008). Thus, different

Table 6. Identified compounds, retention time (RT), molecular masses (MM) and relative percentage (%) of *M. canadensis* MC 05 essential oil.

RT (min)	RI	Identification	% rel.
6.47	997	3-octanol	0.50
7.47	1027	limonene	1.00
8.78	1066	cis-sabinene hydrate	0.50
9.98	1102	linalool	4.50
11.96	1152	Mentone	0.50
12.93	1177	Terpinen-4-ol	0.50
13.77	1198	trans-dihydrocarvone	9.50
14.83	1224	cis-carveol	1.00
15.45	1239	M = 152	1.00
15.96	1251	carvone	75.0
19.17	1328	dihydro carveol acetate	2.00
20.36	1357	Eugenol	0.5
20.59	1362	acetate de cis carveila	1.00
21.38	1382	beta-bourbonene	1.00
22.78	1416	trans-caryophyllene	1.50
24.53	1459	cis-muurolo-4(14),5-diene	0.55
25.25	1477	gamma-muurolene	0.50
25.86	1492	Bicyclogermacrene	0.50
26.90	1519	cis-calamenene	0.50
30.43	1612	<1,10-DI-EPI->cubenool	0.50

Table 7. Identified compounds, retention time (RT), molecular masses (MM) and relative percentage (%) of *M. suaveolens* MC 30 essential oil.

RT (min)	RI	Identification	% rel.
20.71	1365	Piperitenone oxide	28.00
22.05	1398	M = 166	4.00
22.37	1406	Alpha-gurjunene	2.00
24.39	1456	E-beta-farnesene	18.50
25.26	1477	Gamma-muurolene	17.50
26.19	1501	n.i.	1.50
26.94	1520	Delta-cadinene	2.00
29.54	1588	Globulol	7.50
31.90	1652	Alpha-cadinol	2.50
33.18	1687	n.i.	5.00
37.22	1802	2-ethylhexyl-salicylate	1.50
39.82	1881	Homomenthyl-salicylate	3.50
42.66	1969	n.i.	7.50

accesses of *M. arvensis* L. presented different yields of essential oil (CM 24 0.95%; CM 26 0.15%; CM 36 0.54%; CM 46 1.02%; CM 59 0.39%; CM 66 0.17%).

The accessions not yet identified in terms of species (*Mentha* spp.) also demonstrated different rates of essential oil, such as *M. piperita* L., *M. x piperita subsp Citrata* Ehrh, *M. spicata* L., *M. longifolia* L. Huds, *Mentha*

spp. x *M. villosa* Huds, *M. aquatica*, *M. canadensis*, *Mentha x piperita*, *M. suaveolens* and *M. rotundifolia*, as shown in Table 3.

The different yields of essential oil obtained for the accessions of a same species cultivated under similar conditions of climate, soil, nutrition, and irrigation are due to genetic particularities of the plant, these results

Table 8. Identified compounds, retention time (RT), molecular masses (MM) and relative percentage (%) of *M. arvensis* MC 36 essential oil.

RT (min)	RI	Identification	% rel.
3.22	-	n.i.	0.50
6.02	978	1-octen-3-ol	0.50
8.09	1046	beta-ocimene	0.50
10.10	1105	Linalool	35.0
10.39	1112	n.i.	0.50
12.76	1172	cis-pinocanfone	0.50
13.57	1193	alpha-terpineol	12.00
13.73	1197	trans-dihydrocarvone	0.50
15.06	1229	nerol (or cis-geraniol)	1.90
15.65	1243	Carvone	3.00
16.25	1258	Linalool acetate	22.00
20.69	1365	neryl acetate	5.00
21.51	1385	geranyl acetate	7.00
22.03	1397	cis-jasmone	0.50
22.79	1416	trans-caryophyllene	4.00
24.38	1456	E-beta-farnesene	0.50
25.25	1477	gamma-murolene	1.00
26.63	1512	M = 222	0.50
27.99	1548	Elemol	1.00
29.58	1589	M = 222	6.00
29.69	1592	M = 204	0.50
31.05	1629	gamma-eudesmol	0.50
31.71	1647	beta-eudesmol	0.50
31.83	1650	alpha-eudesmol	1.00
32.38	1665	Bulnesol	1.00

Table 9. Identified compounds, retention time (RT), molecular masses (MM) and relative percentage (%) of *M. suaveolens* x *spicata* MC 52 essential oil.

RT (min)	RI	Identification	% rel.
3.22	-	n.i.	0.50
7.46	1027	Limonene	0.50
8.77	1066	cis-sabinene hydrate	1.50
9.94	1101	Linalool	1.00
11.78	1148	para-menth-3-en-ol	1.00
12.85	1175	M = 152	2.00
15.57	1241	Pulegone	52.00
15.66	1244	Carvone	2.00
16.78	1270	M = 150	1.00
19.76	1342	Piperitenone	30.00
20.02	1349	M = 164	1.00
22.76	1415	trans-caryophyllene	1.50
24.38	1456	E-beta-farnesene	1.00
25.26	1478	gamma-murolene	5.00
25.86	1492	bicyclogermacrene	2.00

demonstrate the importance of working with a selection of the best genotypes within one same species and guiding the production according to the purpose intended.

Following the extraction of the essential oils (EOs), EOs were tested for antimicrobial activity against planktonic cells of *Candida* spp. isolates.

The inoculum was first standardized in a RPMI-1640 culture medium, according to the recommendations of the CLSI manual (2002). Minimum inhibitory concentration (MIC) results for EOs are presented in Table 3.

Duarte et al. (2005) suggested, based on the work of Aliannis et al. (2001), that MIC values up to 0.500 mg/ml, from 0.550 to 1.500 mg/ml and above 1.500 mg/ml exhibited respectively, strong, moderate and weak antimicrobial activity. Hence, one could consider that the majority of the crude oils from the plants studied up to the moment are capable of inhibiting yeast growth of the *Candida* genus, demonstrating weak to moderate activity, as can be observed in Table 3.

It is important to point out that oils with no activity up to concentrations of 100 µg/ml will probably not have clinical application, as the majority of antibiotics used have active concentrations of approximately 10 µg/ml. Activity up to concentrations of 100 µg/ml of crude plant material, demonstrates a good potential for practical applications, and depending on the chemical nature of the component responsible for the activity, the subsequent fractioning technique may further improve the quality (Rios et al., 1988).

As aforementioned, most species of *Mentha* evaluated in this study presented MIC values greater or equal to 1 mg/ml. These results are in agreement to the studies of Sartoratto et al. (2004), Duarte et al. (2005) and Mahboubi and Haghi (2008), who evaluated the activity of *Mentha* spp. essential oils against *C. albicans* cells. Duarte et al. (2005) evaluated the antimicrobial properties of *M. arvensis* var. *piperita* L., *M. piperita* L. and *M. pulegium* L., cultivated at CPMA – CPQBA, obtaining the following results: 1.1, 0.6 and 0.74 mg/ml, respectively. Mahboubi and Haghi (2008) however, tested the antimicrobial activity of *M. pulegium*, collected at the Herbarium of Agriculture Department of Iran, and MIC values were equal to 1 mg/ml. These results suggest that the essential oils of *Mentha* spp. usually present moderate antimicrobial activity.

Nevertheless, Mahboubi and Haghi (2008) suggested the use of *M. pulegium* essential oil as an alternative to antibiotics, as there has been undesirable side effects and a growing microbial resistance to the later (Jewtuchowicz et al., 2007; Lee et al., 2007; Rosato et al., 2008). The authors point out that further studies are necessary to evaluate the practical value of these therapeutic applications (Mahboubi and Haghi, 2008), as well as to standardize the experimental techniques, for a broader comparison among studies (Janssen et al., 1987).

The results from the present study show that only some species of *Mentha* can present differences in properties as suggested by several studies (Spirling and Daniels, 2001; Xu et al., 2003; Mahboubi and Haghi, 2008). Thus, antimicrobial properties were different for the accesses of *M. arvensis* L. CM 46 and CM 59, that presented MIC values greater than 1 mg/ml for most strains, and the access CM 36, which presented MIC under 1 mg/ml. The *M. canadensis* CM 05 access also showed different antimicrobial properties, with strong anti-*Candida* activity against all strains, whereas the CM 20 access showed a low to moderate activity. Non-identified species showed similar activity, such as accesses CM 51 and CM 56 and accesses CM 57, CM 62 and CM 63. Accesses CM 70 and CM 72, pertaining to the same species, differed only as to their activity against strain 3A5 (<0.007 and 0.015 mg/ml, respectively).

The oils of *M. spicata* L. (CM 17, CM 33 and CM 41) presented different responses to *C. dubliniensis* strains, whereas *M. longifolia* L. Huds differed only against strain 3A5 (0.046 mg/ml for CM 27 and > 1 mg/ml for CM 40).

Mentha sp. x *M. villosa* Huds differed for strain 26A4 (CM 28 above 1 mg/ml and CM 38, 0.5 mg/ml).

M. piperita indicated the same results for different accessions; the same occurred with *Mentha* x *piperita* subsp. *citrata* Ehrh. according to Table 4. When taken together and analyzed, these results demonstrate the importance of studying different plant accessions or genotypes. Regardless of the species, different accessions may present different antimicrobial properties.

To compare the results obtained between the medicinal plants herein studied and commercially available antimicrobial drugs (fungi), Nistatina (Merck® fungicide) was used to determine the MIC of these compounds for the same *Candida* spp. isolates. Results are demonstrated in Table 5.

The oils showing better antifungal activity *in vitro* against *Candida* spp. by broth microdilution were selected for biofilm assay, in an attempt to test these virulence factors against *Candida albicans*. The essential oils of *Mentha* spp. (CM 05, CM 30, CM 36 and CM 52) showed inhibition less than 50% of the biofilm formation, and only CM 30 and CM 52 revealed destruction of the biofilm in less than 50% in mature biofilms of *C. albicans* (SC 5314). The results clearly show that there was a decrease in adhesion and metabolic activity in the presence of different *Mentha* spp. concentrations essential oils confirming the anti-*Candida* activity of these essential oils. However, the commercial antifungals were more efficient against planktonic cells, biofilm formation and mature biofilm at low concentrations compared to the essential oil. This however, does not avoid the future use of *Mentha* spp. as an antimicrobial agent, as there are several reports in the literature indicating the antimicrobial potential of this species. Research to clarify

the properties of extracts and oils derived from *Mentha* spp. in respect to genetic, biochemical, physiological aspects and more detailed knowledge of their bioactive components are important and deserve to be better investigate. The researchers involved in this field, aim towards the use of natural products, and their bioactive compounds that can replace or at least to act as an adjuvant in synergism with commercial antifungals used today to control these microorganisms.

Based on the results, the oils of *M. canadensis* CM 05, *M. suaveolens* x *spicata* CM 52, *M. suaveolens* CM 30, and *M. arvensis* CM 36, were selected for Gas chromatographic analysis CG-EM (Petrakis et al., 2009) due to the great variation found in the chemical composition of these oils. Different compounds for each species of *Mentha* were found (Figure 1 and Tables 6 and 9) according the Deschamps et al. (2008) and Mkaddem et al. (2009).

The essential oils of *Mentha* revealed a complex mixture of chemical compounds, with the major constituents of these essential oils belonging to the class of the monoterpenes (Mahmoud and Croteau, 2001).

The mechanism of antimicrobial action of the oils is not yet clear. Nevertheless, it has been suggested that the mechanism of action occurs through the rupture of the cell membrane, through the affinity of lipophilic components (Schelz et al., 2006), by the action of the class of terpene compounds (monoterpenes and sesquiterpenes) (Mkaddem et al., 2009). Thus, the identification of the compounds of the essential oils in the present study could contribute to the elucidation of the molecular antimicrobial mechanisms of action in future pharmacological studies.

In the essential oil of CM 52, the major compounds were the cetonic compounds, Pulegone (52.2%) and Piperitenone (29.5%), commonly found in the genus *Mentha* (Sutour et al., 2010), including *M. piperita*. Pulegone is considered toxic due to a hepatic metabolic activation of the P-450 enzyme which liberates menthofuran. For this reason the recommended concentration for the use of this compound in cosmetics is up to 1% (Nair, 2001).

The major compounds of the *M. canadensis* CM 05 oil were carvone (74.8%), linalool (4.84%), and dihydrocarveol acetate (2.04%). Sivropoulou et al. (1995) however found carvone to be the major compound for *M. spicata*. In the present study, the major compound of *M. spicata* CM 30 was piperitenone oxide (28.0%). The major compounds of the *M. arvensis* CM 36 oil were linalool (33.2%), linalool acetate (22.0%) and alpha-terpineol (11.8%). Deschamps et al. (2008) found alpha-terpineol (9.6%) to be the major compound; however they also obtained several non-identified compounds in their analysis.

These data illustrate the need to carry out the

identification of the compounds contained in the essential oils of *Mentha* spp., through CG-EM. Despite pertaining to one same species, oils originated from different accessions may present variations in constituent compounds, and consequently in properties.

The specific metabolism of each genetic material could be suggested to result in differentiated contents of monoterpenes in aromatic species included in the herein study, as can be observed in the accessions of *M. canadensis* CM 05, *M. spicata* CM 30, *M. arvensis* CM 36, and *M. suaveolens* x *spicata* CM 52. The importance of monoterpene content in different species is found in the directly proportional relation to oil yield (Dechamps et al., 2008). As the amount of monoterpenes could also be associated to better antimicrobial results, the study of different seasonal characteristics of the plant is important, to verify in which season there is a greater production of monoterpenes, better yields, and consequently, better antimicrobial properties.

The analysis of the oils from *M. canadensis* CM 05, *M. suaveolens* x *spicata* CM 52, *M. spicata* CM 30, and *M. arvensis* CM 36 suggest additional studies with other microorganisms, including inhibitor virulence factors in germ tubes formation, expression of adhesion proteins and other phytochemical studies, involving the fractioning and other chromatographic analysis of these oils.

The largest quantities of essential oil were obtained from *M. spicata* (MC 31) (1.50%) and *Mentha* spp. (1.45%). Oil analyses indicated the presence of volatile derivatives (terpenes) of alcohols and aldehydes, such as menthone, menthol, linalool, carvone, limonene, piperitenone, gamma murolene and pulegone. Terpene compounds are reported in literature as having the ability to disrupt the membrane integrity of microorganisms compromise their functions. The study of different genotypes of *Mentha* spp. shows that the different species of this genus have different properties among them, and therefore differences in antifungal action, that becomes important to characterize the anti-*Candida* activity of the different species tested. The data presented in this study show that only essential oils of certain *Mentha* species have satisfactory results in antifungal activity against planktonic cells of *Candida* spp. and the biofilm of *Candida albicans*. These results pointed out further studies involving the control of clinical fungal infections being used alone and/or in combination with commercial antifungal agents.

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REFERENCES

- Aliagiannis N, Kalpotzakis E, Mitaku S, Chinou IB, 2001.** Composition and antimicrobial activity of essential oil of two *Origanum* species. *J Agric Food Chem*, 40: 4168-4170.
- Badiee P, Alborzi A, Davarpanah MA, Shakiba E, 2010.** Distributions and antifungal susceptibility of *Candida* species from mucosal sites in HIV positive patients. *Arch Iran Med*, 13: 282-287.
- Bhatia SP, McGinty D, Letizia CS, Api AM, 2008.** Fragrance material review on p-mentha-1,8-dien-7-ol. *Food Chem Toxicol*, 46: Suppl 11:S197-200.
- Bush TM, Rayburn KS, Holloway SW, Sanchez-Yamamoto DS, Allen BL, Lam T, So BK, Tran de H, Greyber ER, Kantor S, Roth LW, 2007.** Adverse interactions between herbal e dietary substances and prescription medications: a clinical survey. *Altern Ther Health Med*, 13: 30-35.
- CLSI, 2002.** Clinical and Laboratorial Standards Institute, norma M27-A2. Método de referência para testes de diluição em caldo para determinação da sensibilidade de leveduras à terapia antifúngica. 22(15), 2ª. ed.
- Dahlén G, Blomquist S, Carlén A, 2009.** A retrospective study on the microbiology in patients with oral complaints and oral mucosal lesions. *Oral Dis*, 15: 265-72.
- Dechamps C, Zanatta JL, Bizzo HB, Oliveira MC, Roswalka RC, 2008.** Avaliação sazonal do rendimento em óleo essencial de espécies de *Mentha*. *Cienc Agrotec*, 32: 725-730.
- Duarte MCT, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C, 2005.** Anti-*Candida* activity of Brazilian medicinal plants. *J Ethnopharmacol*, 97: 305-11.
- Fotedar R, Al-Hedaithy SS, 2004.** Prevalence of *Candida dubliniensis* among germ tube-positive yeasts recovered from the respiratory specimens in HIV-negative patients. *Mycoses*, 47: 150-155.
- Furletti VF, Peixoto ITA, Obando-Pereda GA, Mardegan RC, Sartoratto A, Figueira GM, Duarte RMT, Rehder VLG, Duarte MCT, Höfling JF, 2011.** Action of *Coriandrum sativum* L. essential oil upon oral *Candida albicans* biofilm formation. *Evid Based Complement Alternat Med*, 2011: 985832.
- Gurib-Fakin A, 2006.** Medicinal plants: tradition of yesterday and drugs of tomorrow. *Mol Aspects Med*, 27:1-93.
- Höfling JF, Anibal PC, Obando-Pereda GA, Peixoto ITA, Furletti VF, Foglio MA, Golçalves RB, 2010.** Antimicrobial potential of some plant extracts against *Candida* species. *Braz J Biol*, 70: 1065-1068.
- Hur MH, Park J, Maddock-Jennings W, Kim DO, Lee MS, 2007.** Reduction of mouth malodor and volatile sulphur compounds in intensive care patients using an essential oil mouthwash. *Phytother Res*, 21:641-643.
- Jaikittivong A, Johnson DA, Yeh CK, 1998.** The relationship between salivary histatin levels and oral yeast carriage. *Oral Microbiol Immunol*, 13: 181-187.
- Janssen AM, Schefer JJC, Baerheim SA, 1987.** Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of the tests methods. *Planta Med*, 53:395-398.
- Jewtuchowicz VM, Brusca MI, Mujica MT, Gliosca LA, Finquelievich JL, Lovannitti CA, Rosa AC, 2007.** Subgingival distribution of yeast and their antifungal susceptibility in immunocompetent subjects with and without dental devices. *Acta Odontol Latinoam*, 20:17-22.
- Kim J, Seo SM, Lee SG, Shin SC, Park K, 2008.** Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), oriental sweetgum (*Liquidambar orientalis*), and Valerian (*Valeriana wallichii*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *J Agric Food Chem*, 56(16): 7316-7320.
- Lee SB, Cha KH, Kim SN, Altantsetseg S, Shatar S, Sarangerel O, Nho CW, 2007.** The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms. *J Microbiol*, 45(1): 53-57.
- Mahboubi M, Haghi G, 2008.** Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *J Ethnopharmacol*, 119(2): 325-327.
- Mahmoud SS, Croteau RB, 2001.** Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proc Natl Acad Sci USA*, 98: 8915-8920.
- McKay DL, Blumberg JB, 2006.** A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother Res*, 20: 619-633.
- Mimica-Dukic N, Bozin B, 2008.** *Mentha* L. species (Lamiaceae) as promising sources of bioactive secondary metabolites. *Curr Pharm Des*, 14 (29): 3141-3150.
- Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F, Romdhane M, 2009.** Chemical composition and antimicrobial and antioxidant activities of *Mentha (longifolia* L. and *viridis*) essential oils. *J Food Sci*, 74: 358-363.
- Nair B, 2001.** Final report on the safety assessment of *Mentha piperita* (peppermint) oil, *Mentha piperita* (peppermint) leaf extract, *Mentha piperita* (peppermint) leaf, and *Mentha piperita* (peppermint) leaf water. *Int J Toxicol*, 20 Suppl 3:61-73.
- Niimi M, Firth NA, Cannon RD, 2010.** Antifungal drug resistance of oral fungi. *Odontology*, 98:15-25.
- Nogueira JC, Diniz MdeF, Lima EO, 2008.** *In vitro* antimicrobial activity of plants in Acute Otitis Externa. *Braz J Otorhinolaryngol*, 74(1): 118-124.
- Oumzil H, Ghoulami S, Rhajaoui M, Ildrissi A, Fkih-Tetouani Faid M, Benjouad A, 2002.** Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. *Phytother Res*, 16: 727-731.
- Perea S, Lopez-Ribot JL, Kirkpatrick WR, McAtee RK, Santillán RA, Martinez M, Calabrese D, Sanglard D, Patterson TF, 2001.** Prevalence of molecular mechanisms os resistance to azole antifungal agents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother*, 45: 2676-2684.
- Petrakis EA, Kimbaris AC, Pappas CS, Tarantilis PA, Polissiou MG, 2009.** Quantitative determination of pulegone in pennyroyal oil by FT-IR spectroscopy. *J Agric Food Chem*, 57: 10044-10048.
- Prashar A, Hili P, Veness RG, Evans CS, 2003.** Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. *Phytochemistry*, 63: 569-575.
- Rios JL, Recio MC, Villar A, 1988.** Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol*. 23: 127-49.
- Rosato A, Vitali C, Gallo D, Balenzano L, Mallamaci R, 2008.** The inhibition of *Candida* species by selected essential oils and their synergism with amphotericin B. *Phytomedicine*, 15: 66335-66338.
- Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG, 2004.** Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz J Microbiol*, 35:275-80.
- Scholz Z, Molnar J, Hohmann J, 2006.** Antimicrobial and antiplasmod activities of essential oils. *Fitoterapia*, 77: 279-285.
- Sivropoulou A, Kokkimi S, Lanaras T, Arsenakis M, 1995.** Antimicrobial activity of mint essential oils. *J Agric Food Chem*, 43: 2384-2388.
- Spirling LI, Daniels IR, 2001.** Botanical perspectives on health peppermint: more than just an after-dinner mint. *J R Soc Health*, 121: 62-63.
- Sullivan DJ, Moran GP, Coleman DC, 2005.** *Candida dubliniensis*: ten years on. *FEMS Microbiol Lett*, 253: 9-17.
- Sutour S, Bradesi P, Casanova J, Tomi F, 2010.** Composition and chemical variability of *Mentha suaveolens* ssp. *suaveolens* and *M. suaveolens* ssp. *insularis* from Corsica. *Chem Biodiver*, 7: 1002-1008.
- Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Falcioni L, Cioni PL, Morelli I, 2005.** The inhibition of *Candida albicans* by selected

- essential oils and their major components. *Mycopathologia*, 159: 339-345.
- Wilson** LS, Reys CM, Stolpman M, Speckman J, Allen K, Beney J, **2002**. The direct cost and incidence of systemic fungal infections. *Value Health*, 5: 26-34.
- Xu** J, Boyd CM, Livingston E, Meyer W, Madden JF, Mitchell TG, **1999**. Species and genotypic diversities and similarities of pathogenic yeasts colonizing women. *J Clin Microbiol*, 37: 3835-3843.
- Xu** P, Jia W, Bi L, Liu X, Zhao Y, **2003**. Studies on components and quality of essential oil from *Mentha piperita* L. produced in Xinjiang, China. *Chem Ind Forest Prod*, 23: 43-45.
- Zapata** B, Durán C, Stashenko E, Betancur-Galvis L, Mesa-Arango AC, **2010**. Antifungal activity, cytotoxicity and composition of essential oils from the *Asteraceae* plant family. *Rev Iberoam Micol*, 27: 101-103.

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